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STUDIES IN CESTODE CYTOLOGY*

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I. INTRODUCTION

In opposition to the chromosome theory of heredity, or at least in question of it, stand most of the few existing reports on the cytology of cestodes. In a series of papers Child (1904 et seq.) attempted to show the frequent occurrence of amitosis in cestode nuclei. He described and figured stages of amitosis, showing nuclei partially constricted while in an apparent resting stage. He figured also, as illustrative of the result of amitosis, nuclei of the same size closely adjacent to each other. Thus, by the method of finding cells which represented all stages in the hypothetical process, Child claimed the existence of the process. He admitted the existence of regular mitosis in cestodes, but denied that it could be demonstrated frequently enough to support the view that it was common. Richards (1911) called attention to the assumption inherent in Child's evidence, denied that Child's "stages of amitosis" were necessarily anything but selected chance deformations and orientations of resting nuclei, and, finally, showed the regular occurrence of mitosis and meiosis in gametogenesis and cleavage of the same cestode, *Moniezia expansa* (Rudolphi), upon which Child had based his hypothesis. Child (1911) responded by publishing his own interpretations of Richards' slides, which, he said, actually supported his own view. Harman (1913) described gametogenesis in another cestode, *Taenia pisiformis* Bloch, supporting Richards' conclusions that cestode nuclei, at least in gametogenesis, divide mitotically. Neither Richards nor Harman presented any evidence of the continuity of the chromosome complement throughout growth; they failed to give the number or morphology of the chromosomes of their respective species. Thus their arguments were open to the objection that amitosis might have functioned in the somatic divisions preceding differentiation of the gonads. Both Harman and Richards figure variation in numbers and kinds of chromosomes (which would, of course, result from the crude process of amitosis). Young (1910) described the somatic nuclei of twelve larval or mature cestodes, concluding that, in all of these worms, nuclei normally develop from cytoplasmic granules. Young (1912) stated (p. 399): "In a total of 33,930 nuclei counted in the Cestode soma, mainly in actively growing regions, I have observed only 50 mitoses. . . . Many cases of probable amitosis occur." Young (1923) described gametogenesis in cestodes, indicating that in his opinion the division and maturation of even the repro-

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ductive nuclei is extremely irregular. Young here (p. 435) advanced the "theory that mitosis is degenerating" in the cestodes. Since this paper only one work has been published on cestode cytology, Motomura's (1929) report of gametogenesis in the paedogenetic cestode *Archigetes appendiculatus* Ratzel. This work gives the chromosome number, describes a conventional meiosis and mitosis, and contains no suggestion that *Archigetes* differs, in its method of nuclear division, from other higher organisms. The fact that *Archigetes* is a rather anomalous form, being a monozoic or "unsegmented" cestode parasite of an invertebrate, makes Motomura's contribution not especially pertinent to the discussion of cestode cytology in general. The succeeding studies are an attempt to apply modern cytological methods to the problem.

II. TECHNICAL METHODS

Prior workers' methods having yielded, on the whole, unsatisfactory results, it was necessary to test these methods, as well as try other methods in common use by cytologists. Several strobilae of the same species, from the same host individual, were considered, for trial purposes, to be practically identical. The effects of fixation of these cestodes severally in various fixing solutions were studied and compared, and the fixative which proved best by this comparison was then used in other tests. Where only one strobila was recovered from a host, it was cut up, in saline solution, into several parts, and the parts fixed in different solutions. Both methods gave useful results. See Table 1.

Smear Methods

Painter's (1939) technique, using acetocarmine on Carnoy-fixed material torn up with steel needles and pressed under a coverglass, with heat, was successful in making tentative chromosome-number determinations. It is not suitable for critical work. The Feulgen reagent, used with Heitz's "Quetschmethode" as suggested by Bhaduri and Semmens (1942), gives sharper stain and better final fixation after Carnoy than does acetocarmine. Fixed material, in water, is mordanted three hours in 1% chromic acid. Rinsed, hydrolyzed in normal HCl at 40° C. 60–80 minutes, the worms are again rinsed, then stained in the Feulgen reagent. Then the worms are cleared in 50% glacial acetic; selected regions are removed, macerated, and pressed under a cover glass. If properly made, as seen under oil, the smear may be made permanent by passage very slowly through 1 : 1 glacial acetic ethanol, the cover removed, dehydrated, cleared, and re-mounted. Counterstaining for nucleoli (see Bhaduri and Semmens, 1942) is desirable.

Sections

Fixation for sectioning was done by the following reagents. Carnoy's, either 3 : 1 alcohol-acetic, or 6 : 3 : 1 alcohol-acetic-chloroform, is a good general fixative, to be followed preferably by the Feulgen reagent or iron haematoxylin. (It is also good for total mount preparations, where identification depends upon those.) Navashin's chrom-acetic-formalin, as used by Husted and Burch (1943) on chromosomes of snails, was not successful, tending to clump the chromosomes. Flemming's osmic acid fixatives, both the "medium" solution and LaCour's 2BE (see LaCour, 1937), gave the best fixation of chromosomes. Of these, the former has little penetrating power, the latter is best for cleavage stages in embryos. Fixatives containing sodium diuranate (Bhaduri and Semmens, 1942), tried upon *Hymenolepis fraterna*

only, gave excellent results, justifying further use. For other fixatives tried, see Table 1.

Dehydration through an ethanol series into chloroform, with slow embedding in paraffin, resulted in successful preparations. See LaCour (1931).

Sections were cut thick enough so that several layers of whole nuclei could be in one section. For cestodes, 14 μ, in general, is satisfactory. Thinner sections may contain valuable mitotic or meiotic figures that have been ruined. Reconstruction of chromosome complements (see Richards, 1911; Pennypacker, 1940; Rees, 1939) is cytologically untrustworthy, and may be avoided by thick sections.

Sections may be stained with crystal violet (Newton and Darlington, 1929), the Feulgen reagent, or iron haematoxylin. The first is not permanent, nor does it follow Carnoy's successfully. The third is more difficult than the others, because the differentiation cannot be easily observed and controlled. The second, therefore, seems the best general stain for cestode chromosomes, since, once the technique is mastered, it is applicable to most material.[1]

TABLE 1. *Summary of fixation trials*

	No. trials	Satisfactory	Unsatisfactory
Carnoy (6:3:1) or (3:1)	15	14	1
Medium Flemming	15	10	5
Strong Flemming	5	2	3
LaCour 2BE	9	6	3
Bouin	1	0	1
Allen B15	2	0	2
Webber's Navashin*	1	1	0
San Felice Navashin	3	2	1
Sodium diuranate (2 formulas)	2	2	0
Totals	53	37	16

* Tissue fixation was so poor as to make identification impossible.

III. CHROMOSOMES OF THE FAMILY HYMENOLEPIDIDAE

The 10-diploid Hymenolepididae

1. *Protogynella blarinae* Jones, 1943 (host, *Blarina brevicauda* Say, 1823), may have in its neck region plentiful mitoses, which fix well with Medium Flemming. The chromosomes (Fig. 2) may be easily classified as five pairs, supposedly homologous. The idiogram, showing one large pair, two middle-sized pairs, and two smaller pairs, was drawn from a study of ten clear mitoses.

The heteromorphic, or "tailed" chromosome shown in the third pair of the figure is characteristic of the members of the species studied. It may be considered, perhaps, to consist of a portion normally condensed at metaphase, together with another portion only partially condensed.[2]

[1] The time of hydrolysis, at 40° C., may usually be 60 minutes. Some evidence of specificity for optimum period of hydrolysis appeared during tests upon 16 cestodes, indicating that an individual optimum might be determinable for an individual species being investigated.

[2] Such a condition, which seems the simplest interpretation in line with described phenomena, is found in the Y chromosome of certain higher organisms, e.g., *Rattus norvegicus* and certain other mammals. (See Koller and Darlington, 1934.) Therefore, the heteromorphic chromosome suggests by analogy a sex-determining mechanism. The cestode in which it is found was described (see Jones. 1943a) as strongly protogynous, a condition, rare in the cestodes, which may be a step toward the dioecious condition found in cestodes of the genus *Dioicocestus* Fuhrmann. If protogyny were dependent upon factors in the heteropycnotic arm of the dimorphic chromosome, a sex-determining mechanism would indeed be present. Two kinds of gametes would be produced, and a progeny segregating for protogyny and a hypothetical protandry could arise. The writer believes that life history and breeding experiments on these cestodes should be undertaken.

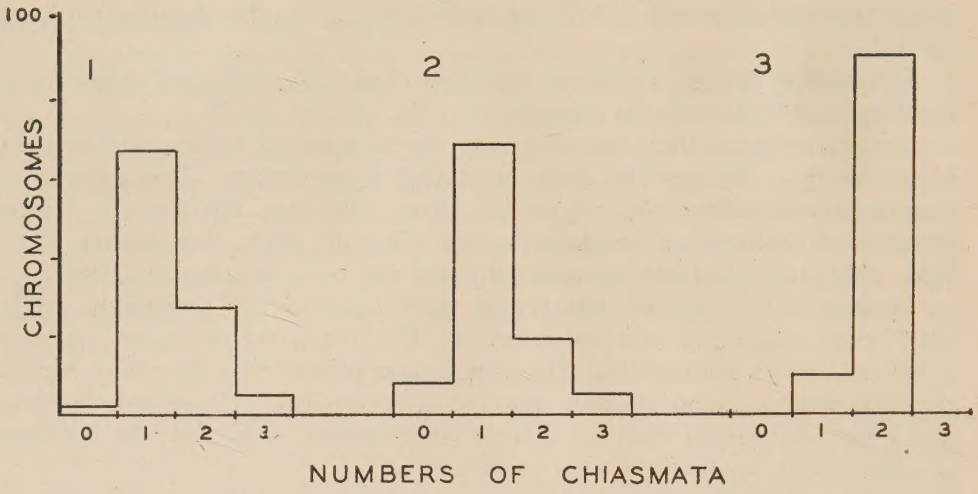

FIG. 1. Chiasma frequencies in three species of cestodes, *Hymenolepis anthocephalus* (1), *Diorchis reynoldsi* (2), and *Hymenolepis serpentulus "sturni"* (3). Chiasmata were counted at diplotene or early diakinesis in each case. Numbers of chromosomes partaking in the formation of no, one, two, and three (the maximum number observed in any bivalent) chiasmata are plotted against the numbers of chiasmata per bivalent.

2. *Diorchis reynoldsi* Jones, 1944, is a co-parasite with the preceding cestode, *Protogynella blarinae*. Its chromosomes (Fig. 3) are somewhat larger than those of *P. blarinae,* but, like them, consist of five pairs. The largest pair has submedian centromeres. The next pair, nearly as long as the first, has subterminal centromeres, as do the three smaller pairs. The chromosomes resemble those of *Hymenolepis fraterna* (Fig. 5) more than they do those of the other members of the family.

In a few preparations diplotene configurations were found, and the number of chiasmata could be counted for each bivalent (Fig. 1). *Diorchis reynoldsi* was found to have a chiasma frequency of 1.23 per bivalent, based on diplotene data. Table 2 compares the chiasma frequencies of five cestodes, including *D. reynoldsi.*

In *D. reynoldsi* meiotic figures showing more than the five bivalents expected on the basis of the mitotic number could be explained as the results of failure of chiasmata. Meiotic nuclei which had extra "bivalents" usually had two distinctly smaller ones (oriented on the spindle, however) plus four large ones; while in plates of five bivalents the two small ones were replaced by a larger one of approximately the same size as the other four. The extra "bivalents" were thus seen to be univalents. These become orientated upon the spindle (unlike most univalents in other organisms), but do not divide at the first division of meiosis. Secondary

TABLE 2.—*Chiasma frequencies in five hymenolepidid cestodes*

Species	No. bivalents	No. chiasmata	Chiasmata per bivalent
Hymenolepis anthocephalus	419	624	1.49
Diorchis reynoldsi	73	90	1.23
H. serpentulus sturni	642	644	1.01*
H. s. turdi	438	500	1.07
H. fraterna	2290	2146	0.94*

* These determinations are minimum frequencies based on study of meiotic metaphases, on the assumption that at least one chiasma must be found in each bivalent.

spermatocytes were found containing six or four chromosomes as well as the usual five, indicating that the univalents pass at random to the poles.

Five different strobilae of *D. reynoldsi* were studied and compared to see how regular was the occurrence of univalents. Table 3 shows that while the average proportion of cells showing complete pairing to those showing two or more univalents is 3 : 1 ; the strobilae vary considerably around this figure. A statistical analysis, using the Probable Error of the ratios and of the mean ratio, shows that most of the variations among these values can be explained as due to sampling error (i.e., the differences are usually less than three times their P.E.; Snyder, 1940, p. 336). It is probable, therefore, that the amount of univalent occurrence in *D. reynoldsi* is a chance phenomenon, connected with the random formation of chiasmata, and is not a constant character for any worm as against another. Univalent frequencies in the individual strobilae then appear as reflections of varying chiasma frequencies. (The latter, it is believed by some, may be subject to environmental or other influences not discernible or classifiable in this material. See Darlington, 1937, page

TABLE 3.—*Univalent frequencies in five strobilae of* Diorchis reynoldsi

Strobila	Numbers of nuclei in		Ratio $\frac{\text{Class I}}{\text{Class II}} \pm$ P.E.*	Significance of difference		
	Class I 4 II + 2 I	Class II 5 II		Strobila	Difference ± P.E.†	Difference / P.E.
1	3	12	0.25 ± 0.097	3–1	0.35 ± 0.11	3.2
2	0	4	... ...	1–4	0.09 ± 0.09	1.0
3	6	10	0.60 ± 0.083	5–1	0.20 ± 0.09	2.2
4	4	25	0.16 ± 0.046	3–4	0.34 ± 0.10	3.4
5	19	48	0.45 ± 0.046	3–5	0.15 ± 0.10	1.5
				5–4	0.29 ± 0.06	4.8
T	32	93	0.344 ± 0.0286	T–1	0.09 ± 0.08	1.1
				T–4	0.18 ± 0.05	3.6
				3–T	0.24 ± 0.09	2.7
				5–T	0.11 ± 0.05	2.2

* P.E. of a proportion, $p = \pm 0.6745 \sqrt{\frac{p\ (1-p)}{n}}$

† P.E. of a difference $= \pm \sqrt{(\text{P.E.}_1)^2 \pm (\text{P.E.}_2)^2}$.

168.) The univalent frequency of strobila 4, as seen in table 3, perhaps differs significantly from that of the other strobilae, except strobila 1, as well as from the total frequency. Since all strobilae here included came from the same host individual, environmental conditions may be assumed the same for all. Whether or not the divergent univalent frequency of strobila 4 is due to genotypic control is impossible to say. This matter will be taken up in the discussion of *Hymenolepis fraterna.*

3. *Hymenolepis fraterna* Stiles, 1906, obtained from the rat, has ten chromosomes in the mitotic complement (Fig. 5). One pair, the largest, has median centromeres. The rest have subterminal centromeres. Besides the pair of large chromosomes, there are two pairs of middle-sized, and two pairs of small. There is a marked similarity, noted above, between the chromosomes of *H. fraterna* and those of *Diorchis reynoldsi.*

The chiasma frequency of *H. fraterna* could be found only as a function of the univalent frequency (Table 4). Diplotene stages were not well preserved by any of the fixatives used. While it is recognized that the relation between low chiasma frequency and univalent occurrence may not be as simple as a mathematical function, yet one may make the assumption for limited purposes of comparison. (Table 2

illustrates simply the differences and similarities of certain closely related cestodes in terms of a character, chiasma frequency, which may, or may not, have actual taxonomic value. The compilation of many more cytological tables, like Tables 2, 3 and 4 herein, might, indeed, establish such a value in chiasma frequencies.) The chiasma frequency of *H. fraterna,* on the assumption of at least one chiasma per bivalent to give complete pairing, is ca. 0.95 chiasma per bivalent. This value is derived from study of three strobilae (460 cells) from a single host.

The univalent frequencies in these three strobilae appear to vary over a considerable range (Table 4). By computing the Probable Error for proportions, and deriving the P.E. of differences (see Snyder, 1940: 336, 337), it was determined that the differences between most of the ratios are probably not due to sampling error alone. It can therefore be said that univalent frequencies do vary, both as between different strobilae, and as between different sexes in the observed specimens of *Hymenolepis fraterna.*

TABLE 4.—*Univalent frequencies in spermatocytes and oöcytes of three strobilae of* Hymenolepis fraterna

Strobila and sex of tissue	Numbers of nuclei in		Ratio	Significance of difference		
	Class I 4 II + 2 I	Class II 5 II	$\frac{\text{Class I}}{\text{Class II}} \pm$ P.E.*	Tissues and strobilae	Difference ± P.E.	$\frac{\text{Difference}}{\text{P.E.}}$
♂ a	37	66	0.56 ± 0.033	♂ a – ♂ b	0.16 ± 0.046	3.5
♂ b	30	75	0.40 ± 0.032	♂ c – ♂ a	0.26 ± 0.053	4.9
♂ c	18	22	0.82 ± 0.041	♂ c – ♂ b	0.42 ± 0.054	7.8
Total	85	163	0.520 ± 0.0233			
				♂ a – ♀ b	0.22 ± 0.046	4.8
♀ a	No data	...	.....	♂ a – ♀ c	0.27 ± 0.046	5.9
♀ b	26	76	0.34 ± 0.032	♂ b – ♀ b	0.06 ± 0.045	1.3
♀ c	23	79	0.29 ± 0.031	♂ b – ♀ c	0.11 ± 0.045	2.4
Total	49	155	0.316 ± 0.0214	♂ c – ♀ b	0.48 ± 0.045	10.0
				♂ c – ♀ c	0.53 ± 0.052	10.0
♂ ♀ b	56	151	0.370 ± 0.0212			
♂ ♀ c	31	101	0.308 ± 0.0272	♀ b – ♀ c	0.05 ± 0.045	1.1
				♂ T – ♀ T	0.204 ± 0.030	6.8
				♂♀ b – ♂♀ c	0.062 ± 0.0313	1.9

* See footnote to Table 3.

Univalent occurrence affected zygotic chromosome number in this cestode. The first cleavage of at least two out of eight eggs (selected only for clearness of division figure) showed a diploid complement of twelve, two more than the somatic complement of the parent. It may be supposed that two gametes, each with six chromosomes, fused to form such zygotes. Such gametes might frequently arise in strobilae whose meiotic divisions show a high univalent frequency, considering the known tendency of univalents in other material to segregate irregularly to the daughter nuclei of the first division. This possibility was suggested as an explanation for observed chromosome number variation in a turbellarian; Jones, 1943b.) A broad survey of chromosome number in *Hymenolepis fraterna* might reveal the survival as adults of the 12-diploid zygotes.[3]

[3] The problem of variation in cestodes has received both taxonomic (e.g., Venard, 1938) and physiological (e.g., Larsh, 1943 et seq.) attention. The conclusions reached in regard to the problem cannot be considered finally valid, however, until some attention be given the questions of genetic uniformity and stability in the species or strains considered. The author's finding of frequent zygotic variation in the early embryos within the uteri of a single strobila of *Hymenolepis fraterna* (or *H. nana* var. *fraterna,* the form with which Larsh, 1943 et seq., experimented) suggests the possibility of heterozygosity existing in a rigidly selected "strain."

4. *Diorchis ralli* Jones, 1944, from the King Rail, has a diploid complement of 10. Unlike the preceding worms, it has (Fig. 4) two pairs with median centromeres, one large, the other, middle-sized. There is one intermediate pair with subterminal centromeres, and there are two small pairs in which the centromeric constriction is not evident. In meiosis, good evidence was found for the occurrence of univalents. Metaphase I of meiosis is shown in the second figure (Fig. 4), with univalent chromosomes near the poles of the spindle, and four bivalents orientated at the equator.

The 12-diploid Hymenolepididae

1. *Hymenolepis anthocephalus* van Gundy, 1936, has 12 chromosomes, consisting of two large pairs (one with submedian centromeres and one with subterminal centromeres), and of four small pairs. The chromosomes of this cestode resemble those of *Diorchis reynoldsi* (Fig. 3) more than any other of the group.

H. anthocephalus presented further opportunity for chiasma study, its meiotic prophases being exceptionally well preserved. About 420 bivalents at diplotene were studied and the number of chiasmata counted. As appears from Fig. 1, the curve of distribution of chiasmata is quite similar, in *H. anthocephalus,* to the curve for *D. reynoldsi,* whose chromosomes show similarity to those of *H. anthocephalus.* The relative absence of univalents, with the corresponding increase in the number of bivalents with more than one chiasma, reflects the higher frequency *per* bivalent found in *H. anthocephalus* (see Table 2). On the basis of meiotic pairing, then, *H. anthocephalus* may be considered a more stable organism than either *D. reynoldsi* or *H. fraterna.*

Chromosome aberrations in *Hymenolepis anthocephalus*

In one strobila (which morphologically resembled other strobilae of *Hymenolepis anthocephalus*) a radical irregularity in chromosome behavior occurred. This was the presence of ring chromosomes in the somatic mitoses (with the apparent consequences of this aberration) traceable all the way from the proliferating neck to the developing gametes.

In the somatic mitoses of the aberrant cestode are frequent ring and dicentric chromosomes at metaphase, and chromosome bridges at anaphase and telophase (see Fig. 10). Chromosome counts of eight, nine, ten, and occasionally more, can be made from somatic metaphases. The number twelve, normal for the species, is rare. The lengths of the chromosomes, and the sizes of rings, vary noticeably in neighboring nuclei. At anaphase, bridges are strikingly frequent; of one hundred anaphases or early telophases picked at random, about sixty had unmistakable bridges. Others showed evidence of breakage of an early bridge. Five or six anaphases had two bridges, involving, therefore, two or more chromosomes. The "normal," or bridgeless, anaphases were, therefore, exceptional.

In meiotic prophases rather complex configurations, capable of several interpretations, appear (see Fig. 10, J). According to their apparent structure and

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Physiological or morphological variation in the strain should not then be attributed solely to environmental differences supplied by different experimental hosts, but may be attributed also to the selective effect of such environments. Continued breeding of the selected strains in the different hosts, coupled with cytological examination of the cestodes, could eliminate the uncertainty inherent in Larsh's (1943 et seq.) and Venard's (1938) experiments.

FIGS. 2–5. Description: All figures ca. 4300×

FIG. 2. *Protogynella blarinae:* Mitosis, showing ten chromosomes, including the heteromorphic one; meiosis, metaphase I, showing five bivalents; and an idiogram* for the species, derived from estimated measurements of ten metaphase plates.

* The word idiogram is used in this paper not in its technical sense, but in the sense of a convenient arrangement of depicted chromosomes, for the purpose of illustrating chromosome morphology.

orientation, they are multivalent associations of chromosomes. While they are too small to show chiasmata in detail, they may have what looks like a triple chiasma involving close end-to-end association of three homologues in the pattern known as "ring-and-rod trivalent" in the well known *Datura* configuration, or they may be more complex, like the "quadrivalent" drawn, diagrammed, and photographically reproduced in Fig. 10, G, which seems to involve four chromosomes. In meiotic metaphase I, the ring-rod configurations persist, and assume a conventional appearance, except that the ring constitutent may be disproportionate in size to the rod. More than one ring-rod may be present in one nucleus. Unfortunately, the inconstancy of chromosome number in the premeiotic figures makes it impossible, by a count of the other chromosomes in the nucleus, to ascertain how many chromosomes actually take part in those associations.

The aberrations reported above may be accounted for by McClintock's (1932 et seq.) clear description of the way in which ring chromosomes behave. In maize, ring chromosomes at mitosis may divide in one plane, in two, or in several. Thus, at mitotic anaphase, identical freely separating rings may result from division in one plane, a "double-sized ring chromosome" results from division along two planes, and interlocking rings result from division along three planes. Both double-sized rings and interlocks form chromosome bridges at anaphase, which must break for separate nuclei to form. As McClintock showed in maize, the broken ends reunite, or unite with other broken ends, to form either new rings or dicentric chromosomes in the daughter nuclei. (Dicentric chromosomes may also arise from the same conditions that produce rings.)

The ring-bridge cycle produces great changes in daughter nuclei. Small rings may not be able to separate, if interlocking, and may become thus duplicate in one daughter nucleus and absent in the other. This fact rather easily explains the number variations in somatic chromosomes mentioned above. The fact that most of the nuclei of the aberrant cestode have less than the twelve chromosomes normal for the species suggests the frequent loss in the cytoplasm (as described by McClintock, 1938b) of small ring chromosomes. Mitotic variation in chromosome length (such as that found in the cestode) can be explained on the assumption of irregular breakage of double-sized rings, such rings having grown by continuation of the ring-bridge cycle. The limit of growth for a ring is probably reached when broken ends are too far apart (chromosome has become too large, or the breaks occur too far from each other) for them to reunite. The limit of shrinkage is reached when the interlocking rings are too small fully to separate at anaphase, so that complete loss or inclusion in only one daughter nucleus occurs.

Meiotic configurations are affected, whether the incidence of ring chromosomes is low, as in untreated maize, or high, as in X-rayed maize or the aberrant cestode. From breakage at different places along a ring chromosome at different divisions, the order and number of genes may be shifted, so that the ring chromosome becomes structurally different from its rod homologue. Where two rings exist in a single

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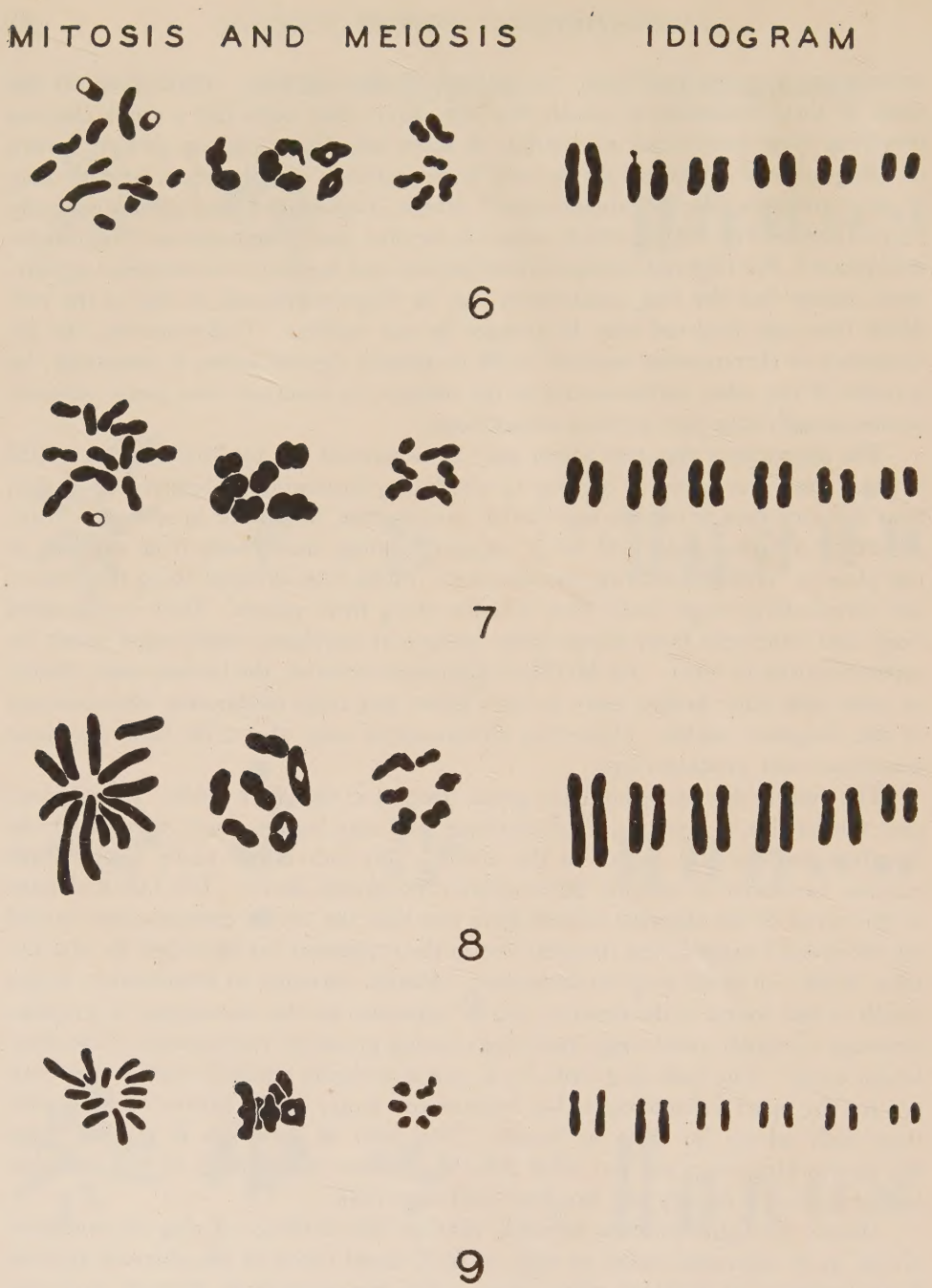
FIG. 3. *Diorchis reynoldsi:* Mitosis, meiosis (I and II), and idiogram derived from several clear mitoses. Four bivalents and two univalents are shown in meiosis I.

FIG. 4. *Diorchis ralli:* Mitosis, meiosis (I and II), and idiogram. Four bivalents and two univalents shown in meiosis I.

FIG. 5. *Hymenolepis fraterna:* Mitosis, meiosis (I and II), and idiogram. Five bivalents shown at meiosis I. Note separate arms of chromosomes at metaphase II.



FIGS. 6–9. Description: All figures ca. 4250×.

FIG. 6. *Aploparaksis sp.:* Mitosis (a late prophase), meiosis I (diakinesis), meiosis II, and idiogram. Note heteromorphic chromosome shown in mitosis and idiogram.

FIG. 7. *Hymenolepis serpentulus sturni:* Mitosis, meiosis I (diakinesis), meiosis II, and idiogram. Note especially the appearance of duplicated pairs of chromosomes; is the base haploid set 3, - - - - two submedians and a subterminal? In this tentative subspecies a few "quadrivalents" were seen.

FIG. 8. *Hymenolepis serpentulus turdi:* Mitosis, meiosis I (diakinesis), meiosis II, and idiogram. Note differences in size and morphology between these chromosomes and those of the other tentative subspecies, Fig. 7.

FIG. 9. *Hymenolepis anthocephalus:* Mitosis, meiosis I (metaphase), meiosis II, and idiogram. Compare this with Fig. 3, noting similarity in morphology between these parasites of the same host.

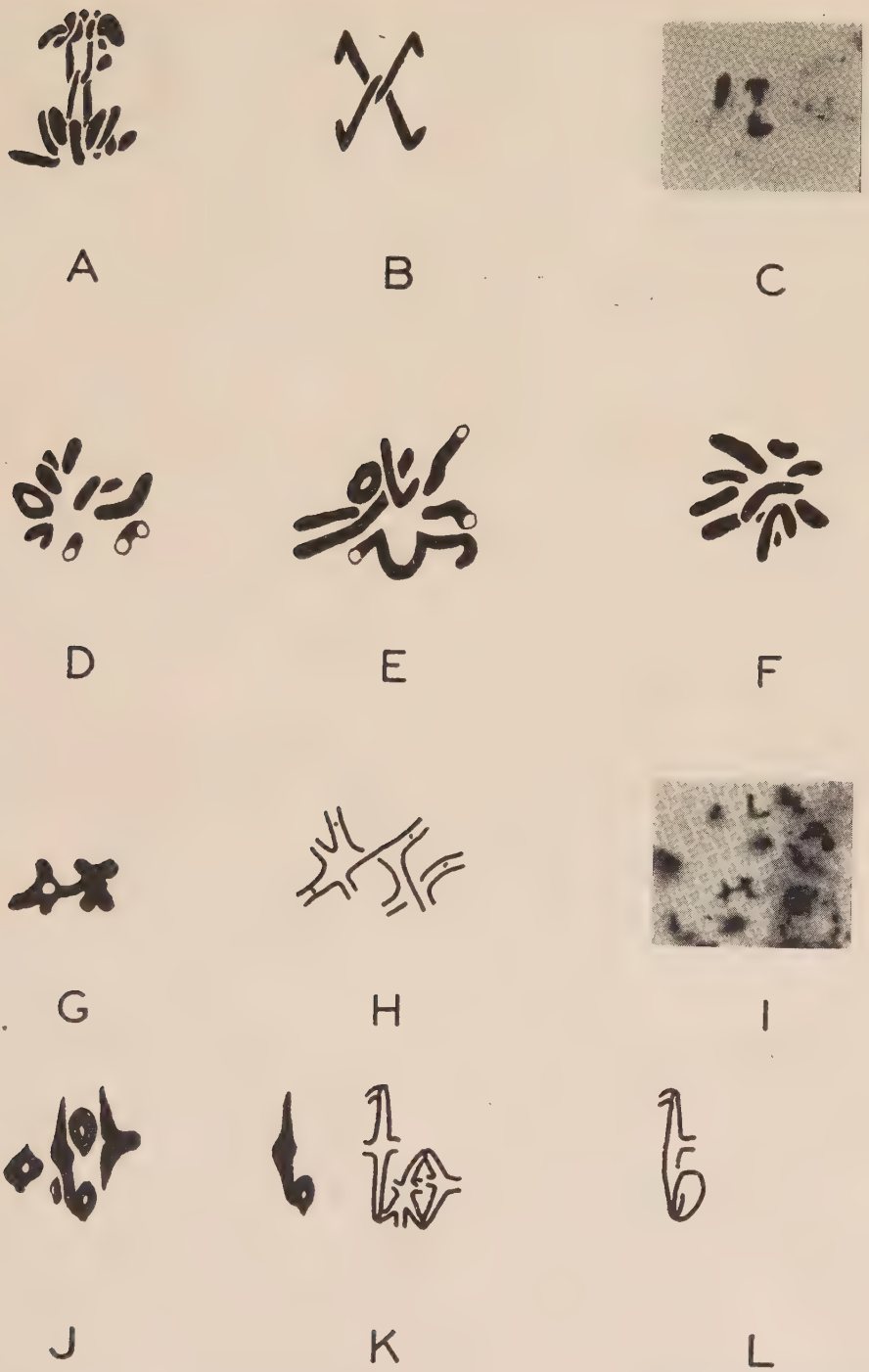


FIG. 10. Description: Aberrant configurations in a strobila of *Hymenolepis anthocephalus* are shown, from top to bottom, in anaphase of mitosis A, metaphase of mitosis D, E, F, diplotene of meiosis G, I, and metaphase of meiosis J. Diagrams illustrate interlocking dicentric chromosomes B, a quadrivalent association at diplotene H, and two possible explanations of the ring-rod configuration at first metaphase K and L. The photographs portray the actual configurations drawn. Magnification of drawings ca. 4800×. Magnification of photographs ca. 2300×.

nucleus, broken ends of one may unite with those of another, forming a dicentric chromosome. When a dicentric chromosome of whatever origin divides at mitosis, it, like a ring, may separate into free and equal parts; or it may interlock in the region between the centromeres, and form a bridge at anaphase. The bridge shown in Fig. 10, A, B, C, is probably of the latter type. Breakage of a dicentric into two chromosomes, by the breaking of such a bridge, will almost certainly leave in one of the parts a segment translocated from the other part. Thus the quadrivalent association shown in Fig. 10, G, may be thought of as the result of one or more such translocations. The ring-rod configuration (Fig. 10, J) seen at first metaphase may be, however, merely the product of simple pairing of a persistent ring with its rod homologue. A single chiasma at pachytene could produce the configuration seen at metaphase (Fig. 10, L).

It is apparent from the foregoing account that the ring chromosomes seen at mitotic metaphase in *Hymenolepis anthocephalus* are probably of the same sort as those seen in maize. The explanation of aberrant chromosomal configurations in maize serves to explain the same kinds of aberrations in the cestode.

2. *Hymenolepis diminuta* (Rud.) Blanchard, 1891, from rats and mice, was very difficult to fix well. It is not represented in the plates because no very good mitoses or meioses could be found. The number of chromosomes is probably 12, but a few rather poor aceto-carmine smears furnished the only mitotic data. The worm can be fixed well, perhaps in the Bhaduri and Semmens fixative, which has not yet been thoroughly tested on cestode material.

3. *Aploparaksis* Clerc. 1903, is a genus represented in this study by a fragment of one cestode, recovered from the intestine of a starling along with several specimens of *Hymenolepis serpentulus* (Schrank) Cohn, 1901. Upon reconstruction of serial sections, this worm (which looked very much like *H. serpentulus*) was found to have but one testis *per* proglottid. Hence it must be placed in the above genus. Further identification could not well be made, as the scolex was missing.

The chromosomes of this cestode look rather like those of *H. serpentulus sturni* (the temporary subspecific is the writer's designation used to distinguish this worm from one of the same species found in the robin, to be discussed later), the cestode with which it was found (Figs. 6, 7). The latter lacks the pair of large chromosomes with median constrictions, as well as the heteromorphic chromosomes found in the *Aploparaksis* species; but the "general morphological similarity" between the chromosomes of both worms, in mitosis and meiosis, is pronounced. The heteromorphic chromosome is not as constant as that found in *Protogynella blarinae* (see above) but appears often enough to be considered characteristic.

4. *Hymenolepis serpentulus* (Schrank) Cohn, 1901, is represented here by several strobilae recovered from the common starling, as well as by several from the robin. Since there are cytological differences between the two forms, as well as slight morphological differences, the worms will be taken up separately. Awaiting a study of the type for the species, and the collection of more specimens from each host, the author proposes, solely for present purposes, to designate the two cestodes by subspecific names.

Hymenolepis serpentulus sturni, the starling's parasite, has four identical pairs (or eight morphologically similar chromosomes) with median centromeres, and two slightly different pairs of smaller chromosomes (Fig. 7). This complement is

unique among the cytologically known HYMENOLEPIDIDAE, in that median constrictions are not elsewhere so frequent.

The chiasma frequency of *H. serpentulus sturni* can be given only as a minimum frequency, based on univalent occurrence. In 6 out of 106 cells studied at meiotic metaphase I, the chromosomes are present as four bivalents plus one quadrivalent. The quadrivalent (if such it is) is always a chain of four chromosomes, except in one case where a ring was found. Considering the doubtful configuration as a quadrivalent,[4] the minimum chiasma frequency becomes 1.01 per bivalent, or 6.06 per cell. It is probable that this figure is near the actual, but the prophase fixation was not good enough to permit analysis of bivalents.

5. *Hymenolepis serpentulus turdi,* the cestode recovered from the robin, is morphologically similar to the cestodes of the same species recovered from the starling. There are, however, differences in dimensions which may have taxonomic significance, if found, from extensive collections, to exist as constant distinguishable features. The rostellar hooks, 10 in number in each cestode, are about 8–10% shorter in the robin cestode; their shape is the same, however, in both subspecies. The scolex of the robin cestode is likewise about 8–10% smaller in its linear dimensions. The neck is more slender. The dimensions of the proglottids are about the same for corresponding regions of the strobila in both. Internal anatomy is not different. The strobila of *H. serpentulus sturni* is probably over all longer than that of *H. s. turdi,* but the given range of variation in Cohn's description probably permits such differences. On the basis of material at hand (eight strobilae from the starling, and four from the robin) it does not seem wise to break down the species, or to describe one of the forms as new. The fact that at least twenty-five separate references to this species occur in the literature (as compiled by Hughes, 1940a) makes the problem of variation within the species an interesting one.

Cytologically, the two cestodes show definite differences. *H. serpentulus sturni* (Fig. 7) has four almost identical pairs of small, medially constricted chromosomes, these being slightly larger than the two other pairs. *H. s. turdi* (Fig. 8) has one large pair with subterminal centromeres, three smaller pairs (still larger than any of the chromosomes of *H. s. sturni*) with subterminal constrictions, a single smaller pair of the same type, and a single pair of chromosomes about the size of the small chromosomes of *H. s. sturni.* This cytological difference is probably significant. Although the size of chromosomes is a variable thing, even within an individual, the morphology of chromosomes, as evidenced by centromere location, is not thought to be variable in genetically homogeneous individuals or populations. When structural diversity is observed between the chromosome complements of two organisms, genetic diversity may be presumed. In the narrowest sense this must be true, whatever the phenotypic evidence, since it is well known that, in many organisms, the mere inversion or translocation of parts of a chromosome is a permanent

[4] It is the writer's opinion that what he sees in this material is an association of more than two chromosomes. But the possibility of fusion artifact must be considered before any conclusion is drawn. Those interested may look again at Fig. 7, and note the appearance of chromosome reduplication, as if a base number of three had become involved in tetraploidy. As White (1940) pointed out in his survey report of the chromosomes of hermaphrodites, there seems to be no reason why hermaphroditic animals should not show the same range of polyploidy found in the higher plants.

change in the organism which may be demonstrated cytologically. It is almost axiomatic that genetic mutation has its seat in the chromosome; thus where chromosomes of otherwise similar organisms differ by structural mutation, one can say that in that respect at least, and also, probably, in physiological respects as well, the two organisms are different. On a cytological basis, therefore, the two types of *H. serpentulus* can be distinguished. But certainly the taxonomic value of cytological data is still very slight in most fields of investigation, in the practical uses to which taxonomy must be put. It might be unwise to attempt formally to split the recognized species into two subspecies or varieties, on the basis of the slight morphological evidence presented in the preceding paragraph, and upon the evidence just presented concerning cytological differences.

Conclusion: the Hymenolepididae

From the detailed description of the chromosomes of nine species from the family HYMENOLEPIDIDAE, a few general conclusions may be drawn. First, so far as the present study goes, cestodes of this family show considerable uniformity in number and type of chromosomes. Fig. 11 is an illustration of how the chromosome numbers and morphologies in the HYMENOLEPIDIDAE are distributed more or less continuously among the taxonomic groups. For added interest, the scoleces, at various magnifications, are sketched beside the chromosomes of each species. This illustration suggests that the HYMENOLEPIDIDAE are a homogeneous group, in which generic divisions are perhaps formal. While it cannot be said, from present data, how far this uniformity extends throughout the whole family, it can be stated with conviction that, cytologically, the representatives studied are similar.

IV. CHROMOSOMES OF THE FAMILY DILEPIDIDAE

Six species from the family DILEPIDIDAE were found suitable for chromosome studies. Three of these belong to the subfamily PARAUTERININAE Fuhrmann and three to the subfamily DIPYLIDIINAE Stiles. (Several other cestodes of this family and of the families ANOPLOCEPHALIDAE Fuhrmann and AMABILIIDAE Braun were obtained; but no accurate chromosome number or determinations on morphology could be made, either because the cestodes were dead when recovered, or because the fixation technique failed.)

1. *Rhabdometra similis* Ransom, 1909, from the hairy woodpecker (a new host record for this cestode), has a diploid complement of 12. There are (see Fig. 12) a large pair with subterminal centromeres, another pair of the same size with median centromeres, a smaller pair with median centromeres and three pairs of still smaller chromosomes.

The chromosomes of *R. similis* are the smallest yet found in the DILEPIDIDAE. Probably those of *Anonchotaenia globata* (?) (Fig. 13) are actually as small; but the figures for the latter were taken from aceto-carmine preparations, in which the chromosomes are generally one-third larger than they appear in other preparations. The structural differences between the chromosomes of *R. similis* and those of *A. globata* are not marked.

2. *Anonchotaenia globata* (von Linstow), Joyeux and Baer, 1936, from the phoebe, is both cytologically and morphologically close to *R. similis*. Its chromosomes (Fig. 13) consist of one large pair with median centromeres, one smaller

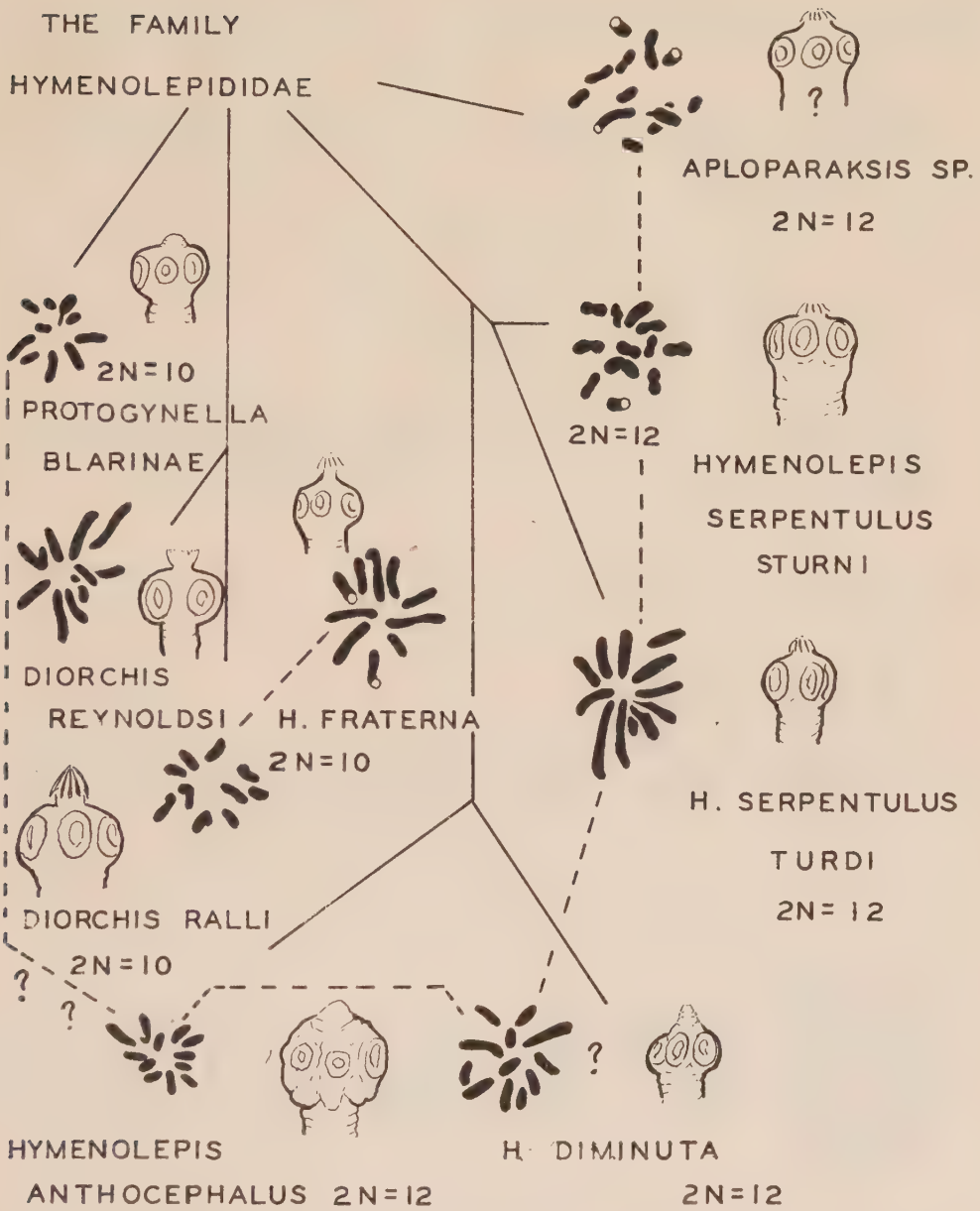


FIG. 11. Description: Four genera of the family are represented by nine complements. The scolex of each worm is sketched beside its chromosomes. Straight lines indicate probable affinities, on morphological grounds. Broken lines indicate probable affinities, on cytological grounds. The line between *D. reynoldsi* and *H. anthocephalus* is highly problematical, suggested by the univalent occurrence in *D. reynoldsi* as a possible means of change in chromosome number. In general, chromosomal and morphological characters overreach generic limits, and support the suggestion that this family is relatively uniform. Magnification of chromosomes ca. 3600×. Magnification of scoleces, 22.5× to 45×.

FIGS. 12–15. Description: All figures ca. 4000×.

FIG. 12. *Rhabdometra similis:* Mitosis, meiosis (I and II) and idiogram.

FIG. 13. *Anonchotaenia globata:* Mitosis and idiogram. This drawing was taken from an aceto-carmine preparation, which probably caused considerable swelling. Fixation in LaCour's 2 BE, which was used for the preparations drawn in Fig. 12, would have resulted in chromosomes of comparable size.

FIG. 14. *Anonchotaenia sp.:* Mitosis (early anaphase), meiosis I (diakinesis), meiosis II, and idiogram. Note the similarity in type to chromosomes of Figs. 12 and 13. The reduplication of four small chromosomes in 12 or 13 would result in a complement like 14.

FIG. 15. *Monopylidium sp.:* Mitosis, meiosis (I and II) and idiogram. Note dissimilarity of this to three preceding, and similarity to Fig. 16.

pair with median centromeres, a middle-sized pair, with subterminal centromeres and three smaller pairs. A comparison of the idiograms in Figs. 12 and 13 shows how alike the two complements are.

The scolex of *A. globata,* illustrated in Fig. 20, is probably similar in form to the normal scolex of *R. similis.* The type for the latter lacked a scolex. A scolex was present in the complete cestode recovered by the author, but it possessed only three suckers, instead of the four found throughout the whole order CYCLOPHYLLIDEA, to which these cestodes certainly belong. Sectioned and stained for histological details, this scolex showed that the fourth sucker was present only as a sclerotic mass of cells lying beneath the cuticula, surrounded by rudimentary protractor and retractor muscles. It is probable that this scolex was "abnormal"; yet its functional parts suggest the hypothetical normal scolex of *R. similis* (as drawn in Fig. 20). A note has been prepared, emending Ransom's description of the species.

3. Four strobilae of another species of *Anonchotaenia* were recovered from a golden-crowned kinglet. This cestode, not yet positively identified as to species, is clearly distinct from *A. globata* because of the unusual vermiform, hookless (?) embryos contained in its ellipsoidal eggs.

Its chromosomes are shown in Fig. 14. They are seen to be 16 in number, with the large medially constricted pair and the other large pair noticeable in the idiograms of the two preceding species (Figs. 12 and 13). In addition to these two pairs, there are six pairs of smaller, not uniform, but hardly distinguishable, chromosomes.

4. A specimen of *Liga brasiliensis* Ransom, 1909, formerly reported (Ransom, 1909) from the golden-winged woodpecker, was recovered from the hairy woodpecker. Its diploid complement of 14 chromosomes was studied mainly in cleavage divisions of the eggs. The morphology of the complement can be seen (Fig. 16) to be similar to that of *Monopylidium sp.* (Fig. 15). Add one pair of the smallest type chromosome to the idiogram of *Liga,* and it will be like that of *Monopylidium,* except for size and the relative lengths of the large subterminals in each species.

5. A single complete worm, identified as to genus as *Choanotaenia* Railliet, 1896 (= *Monopylidium* Fuhrmann, 1899), recovered from the robin, has perhaps the largest chromosomes yet found in the cyclophyllidean cestodes. This worm has a diploid complement of 16. There are (Fig. 15) a large pair with submedian centromeres, a large pair with subterminal centromeres, a slightly smaller pair with submedian centromeres, a still smaller pair with submedian centromeres, and four small pairs with submedian centromeres.

Morphologically, this worm is close to the genus *Liga* (see sketches of respective scoleces in Fig. 20). Its general shape and size are different, and the genital systems are unilateral, not alternating as in *Liga;* but the smaller anatomical details, such as testis number and structure, ova nutrition in parenchymatous capsules, etc., show the morphological relationships to be close. Its chromosome number, sixteen, to *Liga's* fourteen, is a difference rather insignificant when weighed against the marked similarities between the complements.

6. *Dipylidium caninum* (Linné, 1758),[5] the common dog and cat tapeworm,

[5] This cestode species has been the subject of considerable controversy, some workers, e.g., Millzner (1926), stating that the dog-cat tapeworms of this genus constitute many species, others, e.g., Venard (1938), reducing the number of species to one or a few. Since the controversy will resolve itself into merely difference of opinion, unless some new taxonomic criteria

is worth a special study, which the author has not had time to make. The chromosomes (see Fig. 17) probably number 10 diploid. The idiogram is in this case derived solely from meiotic stages, and may, therefore, be inaccurate.

Conclusions: the Dilepididae

The descriptive data given above suggest two tentative conclusions. First, the wide range of chromosome numbers (four) in the DILEPIDIDAE, when compared with the narrow range (two) found in the HYMENOLEPIDIDAE, indicates that the DILEPIDIDAE are cytologically diverse. The conclusion is admittedly based on data whose sampling error may be very large; each group studied represents a very small percentage (ca. 2.0%) of the known numbers of species in the family. But a study of even such small portions of rather well defined groups of organisms will be more likely to reveal a trend which exists in the whole of the groups, than to simulate a trend which does not exist beyond the sample. Second, the chromosomes of the DILEPIDIDAE, as here reported, support rather than question the taxonomic ordering of that family, as regards the two subfamilies DIPYLIDIINAE and PARAUTERININAE. In the first subfamily, the cestodes *Monopylidium* sp. and *Liga brasiliensis* show both morphological and chromosomal affinity. The other member of that subfamily, *Dipylidium caninum,* although its chromosome number is only 10, as against the others' 16 and 14, is probably not out of place, cytotaxonomically, in their company. In the second subfamily, the PARAUTERININAE, the two cestodes *Rhabdometra similis* and *Anonchotaenia globata* are almost identical, cytologically. Their morphological characters are also similar, the main generic difference being that in *R. similis* the genital ducts pass between the excretory canals, while in *Anonchotaenia* they pass dorsal to the canals. The other species of *Anonchotaenia,* while it has four more chromosomes than the other two, and differs from them radically in the embryo stage, shows chromosome structure that follows the idiogrammatic pattern set by them. Figure 20 illustrates the subfamily coherence evinced in both cytological and morphological ways by the cytologically known DILEPIDIDAE.

V. GENERAL CONCLUSIONS AND SUMMARY

The above study suggests that the HYMENOLEPIDIDAE are cytologically uniform, the DILEPIDIDAE cytologically diverse. These qualities are not restricted to the chromosomal aspects of the two groups, but extend to other aspects. First, data obtained from the taxonomic arrangement within the two groups indicates that the HYMENOLEPIDIDAE have few genera but many species, while the DILEPIDIDAE have many genera and few species (see Fig. 21). Since the same taxonomists have studied both groups, and to approximately the same extent, the above taxonomic fact can safely be considered a morphological one; i.e., where generic differences

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can be put to use, a cytological comparison of the disputed species is desirable. Venard (1938) approached the problem intelligently when he undertook to control genetically and physiologically the rearing of many strobilae of *Dipylidium caninum,* in laboratory animals, from the eggs of a single proglottid. As Stunkard suggested to the author, however, Venard failed to eliminate the possibility of obtaining a heterozygous progeny as the result of univalent occurrence, hybridity, or other cytological means of large-scale segregation. The fact that Venard obtained, in the experimental progeny, most of Millzner's species, does not, therefore, conclusively show that the difference between these "species" is due merely to environmental influence. A cytological study of that progeny might have resulted in different conclusions.

FIGS. 16–19. Description: Figures 16–18 ca. 4030×; Fig. 19, ca. 2015×.

FIG. 16. *Liga brasiliensis:* Mitosis (early anaphase), meiosis (I and II), and idiogram. Six bivalents are shown in meiosis (metaphase I), with the seventh bivalent probably already separated. Note similarity in chromosome type to Fig. 15.

FIG. 17. *Dipylidium caninum:* Meiosis (diakinesis), idiogram. The information must be considered tentative only, since little material was examined. The idiogram is derived from meiotic configurations and is not reliable.

FIG. 18. *Oochoristica sp.* (Fam. Anoplocephalidae): Mitosis, meiosis (I and II), and idiogram. An incidental report of chromosome number $2n = 10$.

FIG. 19. *Archigetes appendiculatus* Ratzel: Mitosis, meiosis (I and II), with idiogram. Adapted from Motomura's original drawings. Idiogram derived from Motomura's figures of mitotic metaphases.

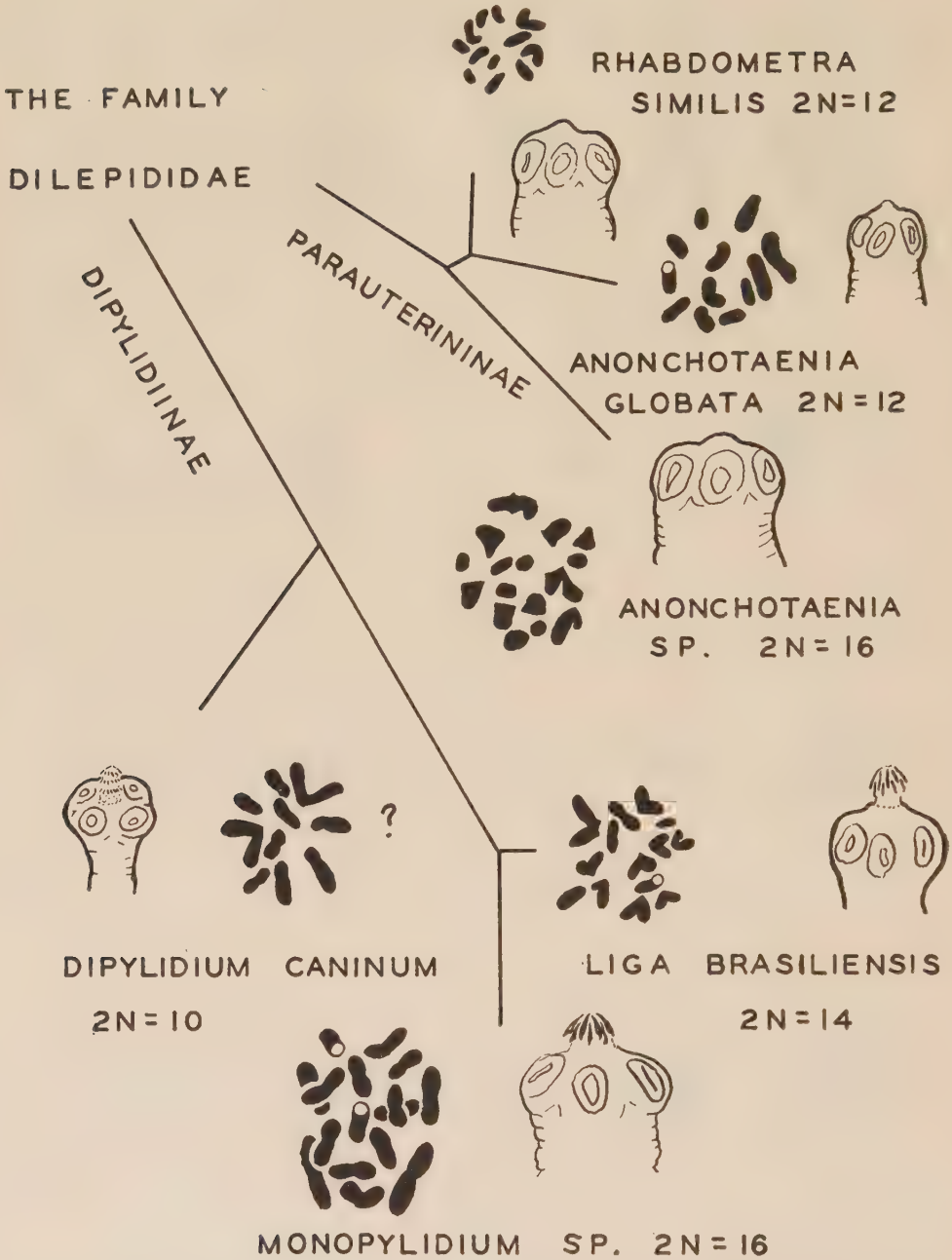

FIG. 20. Description: Five genera of the family are represented by six chromosome complements. The scoleces are sketched beside the chromosomes. Chromosome morphology and number support the taxonomic division into two subfamilies. The type of scolex, especially in respect to the rostellum (armed, in the DIPYLIDIINAE, with two or more rows of hooks), supports the same subdivision. In general, chromosomal and morphological diversity in the family is illustrated. Magnification of chromosomes ca. 3800×. Magnification of scoleces, 23.7× to 47.5×.

are few, as in the HYMENOLEPIDIDAE, the morphology is uniform, while where generic differences are many, as in the DILEPIDIDAE, the morphology is diverse.

Second, ecological data confirm the above cytotaxonomic coincidence. The greater part of the HYMENOLEPIDIDAE (see Hughes, 1940a) are parasites of the avian orders ANSERIFORMES, CHARADRIIFORMES, and COLYMBIFORMES—shore or water birds; while the DILEPIDIDAE (see Joyeux and Baer, 1936) are parasites of the order PASSERIFORMES—land birds of the most varied habits of feeding and nesting. The hosts of the HYMENOLEPIDIDAE, a morphologically uniform group of parasites, are ecologically uniform; while the hosts of the DILEPIDIDAE, morphologically diverse parasites, are ecologically diverse. On ecological grounds, therefore, it may again be said that the HYMENOLEPIDIDAE are uniform, the DILEPIDIDAE, diverse.

FIG. 21. Illustrative graph showing the families of cyclophyllidean cestodes grouped according to numbers of species (dash line) and genera (dot line) with an arbitrary mean or average (dash and dot line). The relative uniformities of the families can be read as functions of the differences between species and genera frequencies. Family 1, MESOCESTOIDIDAE; 2, AMABILIDAE; 3, ACOLEIDAE; 4, DAVAINEIDAE; 5, DILEPIDIDAE; 6, ANOPLOCEPHALIDAE; 7, HYMENOLEPIDIDAE; 8, TETRABOTHRIIDAE; 9, TAENIIDAE; 10, NEMATOTAENIIDAE. (See Hughes, 1940a, 1940b, 1942; Joyeux and Baer, 1936.)

The cytological results of the present partial survey have indicated the same uniformity and diversity. In number and morphology the chromosomes of the hymenolepidid worms are very similar. The idiograms look alike; the numbers found are 10 and 12 diploid, only; and the "general appearance" of the chromosomes of these species shows uniformity. (Chiasma frequency data, since none was obtained from the other family, need not be considered in this respect.) In the DILEPIDIDAE, on the other hand, the chromosomes are varied in number and morphology. Idiograms do not look alike; the numbers found are 10, 12, 14 and 16 diploid; and the "general appearance" of the chromosomes shows diversity. It may be stated, therefore, on the basis of the data at hand, that the chromosome complements of the HYMENOLEPIDIDAE are relatively uniform, while the complements of the DILEPIDIDAE are relatively diverse.

A single hypothesis arises from consideration of the above taxonomic, ecological and cytological conclusions. It is the idea that, in two taxonomic divisions of the cyclophyllidean cestodes, there is coincidence of cytological, ecological, and morphological descriptive data; it is the suggestion that a system of cestode classification may be, by the demonstration of such coincidence, supported as a "natural" system.

SUMMARY

1. The literature on cestode cytology reveals inconclusive results, which in general suggest that cestode nuclei behave differently from nuclei of other organisms. No recent work had been done in this field before the present study.

2. Techniques of collection, fixation, and staining are described. Cestodes, obtained alive from birds, mammals, and amphibians, were found to be best fixed cytologically by acetic-alcohol solutions, osmic-chromic solutions, or sodium diuranate solutions. Sectioning at 14 μ is recommended. Smear or squash technique, with the Feulgen stain, is recommended also. Preferred stains for sections are Newton's crystal-violet-iodine or the Feulgen reagent.

3. The chromosome numbers and morphologies of fifteen cestodes are given. Numbers in the HYMENOLEPIDIDAE are ten and twelve; those in the DILEPIDIDAE are ten, twelve, fourteen and sixteen. An aberration in *Hymenolepis anthocephalus* is described and related to the ring chromosome aberrations in maize. Occurrence of univalents is noted and compared in several cestodes. Chiasma frequencies are reported where possible.

4. Cytologically, the observed portion of the HYMENOLEPIDIDAE is shown to be uniform, while the observed portion of the DILEPIDIDAE is diverse. The families HYMENOLEPIDIDAE and DILEPIDIDAE are discussed in a taxonomic light, on the basis of species- and genera-frequencies, and in an ecological light, on the basis of host uniformity, and are shown to have the same uniformity and diversity established cytologically.

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A NEW ANOPHELINE FROM THE SOLOMON ISLANDS WITH NOTES ON ITS BIOLOGY

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A study of the biologies of the anopheline mosquitoes of the Koli District, Guadalcanal Island, British Solomon Islands Protectorate, revealed the presence of a species that is considered new and is here described under the name *Anopheles koliensis.* A summary of the information on its biology is presented.

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Anopheles koliensis n. sp.

Adult Female.—A medium-sized, yellowish anopheline with a patch of white scales on the ventral surface of the apical third of the labium.

Head: Frontal tuft prominent, white; vertical setae white with admixture of white hair-like scales; top of vertex with white erect scales forming lateral spots, remainder of vertex and occiput covered with erect dark scales. Ocular setae 6. Antenna with minute white scales on third segment. Palpi ornamented as in plate I, markings slightly variable, light scales yellowish to white. Labium black except for patch of white scales on ventral surface of apical third, size of this patch slightly variable, in some specimens visible from above at distal end.

Thorax: Anterior promontory with sparse light scales in center; lateral dense, light with a few dark scales below. Remainder of mesonotum with vestiture of yellowish white scales. Mesonotal bristles golden brown, integument gray with dark eye spot anterior to scutal angle. Pleura dark with evidence of a wide black line dorsally. Spiracular bristle absent, propleurals 4–6, lower sternopleurals 4, upper sternopleurals 6, prealars 8, subalars 7–9, lower mesepimerals absent.

Wing (Plate I): As shown in diagram. Scales rather narrow; pale areas light yellow. Dark spots on costal margin constant; median and subapical dark spots may extend to vein 1. Markings on other veins somewhat variable; distal half of 4th vein darker than other veins; fringe spots constant except dark spot at apex which is variable in size.

Legs (Plate I): Front femora swollen on basal half, uniformly speckled; middle and hind femora speckled, lighter on inner surfaces. All tibia speckled, lighter on inner surfaces. Front tarsus with first segment light at apex and variable number of light spots; second and third segments with basal and apical light bands; fourth with basal light band; fifth dark. First segments of middle and hind tarsi similar to corresponding segment of front tarsus, second, third and fourth segments with small apical light bands, fifth all dark. Second segment of middle and hind tarsi with one or two light spots in middle.

Abdomen: Abdominal vestiture of golden hairs with scales limited to seventh and eighth segments. Seventh segment with few yellow scales. Eighth segment clothed with yellow scales and lateral patch of dark scales at apex. Cerci with yellow scales at base, dark at apex.

Adult Male.—General coloration of the female. Proboscis all black. Fourth segment of antenna with small tuft of narrow white scales. First segment of palpus with few white scales in middle, second with median and apical white bands, third and fourth with apical halves white.

Larva.—*Head* (Plate II): Inner clypeals widely separated, slender, without branches. Outer clypeals unbranched, about two-thirds length of inner. Posterior clypeals small, unbranched. Frontal hairs with branches as shown in plate. Occipitals short, three to four branches. Orbitals minute unbranched. Antenna with minute spines, antennal hair small, unbranched, arising one-third distance from base; terminal hair with three to five branches arising from base.

Thorax (Plate II): Prothoracic hair 1 with small basal tubercle, swollen shaft, six to eight radiating branches on each side, shorter than hair 2. Basal tubercles of 1 and 2 separate. Hair 2 approximately two-thirds length of hair 4, with small tubercle, slender shaft, six to seven branches on each side. Hair 3 simple. Hair 4 with six to eight prominent lateral branches. Hair 5 longer than 4, shaft more slender with short lateral branches. Hair 6 simple, slender, longer than 5. Hair 7 with slender shaft, longer than 6, many long radiating

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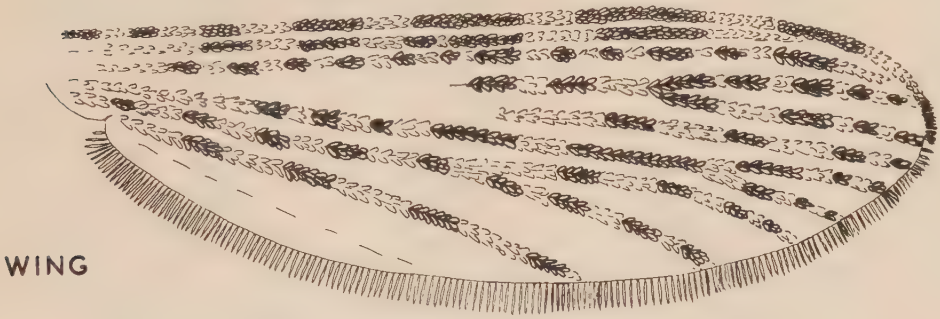
branches. Prothoracic pleural hair 8 long with many branches; 9–12 simple. Mesothoracic hair 1 with thick shaft and eight to ten radiating branches on each side. Metathoracic hair 1 forming a rudimentary palmate hair with five to eight leaflets.

Abdomen (Plate II): Palmate hair on I poorly developed; II to VII well developed (14–16 leaflets); weaker on II. Leaflets (Plate II) pigmented; filaments tapering with two or three indentations on each side. Lateral hairs on III trifurcate, IV and V bifurcate. Anterior tergal plates well developed. Pecten (Plate II) with usually thirteen spines, three or four of these long, remainder medium in length; fine serrations at base of spines. Pecten hair with four or five branches.



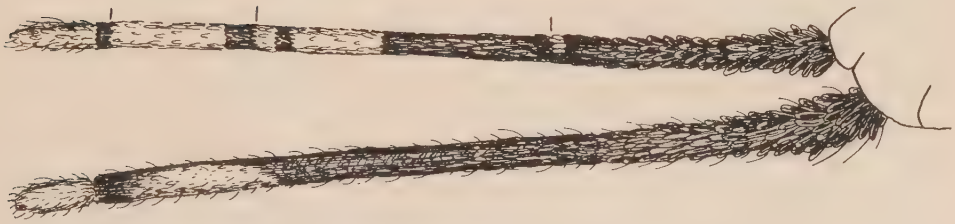
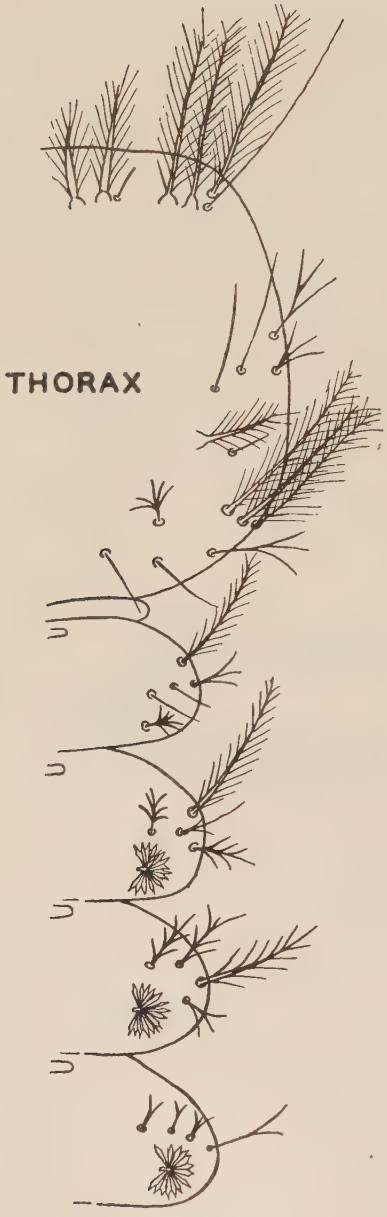
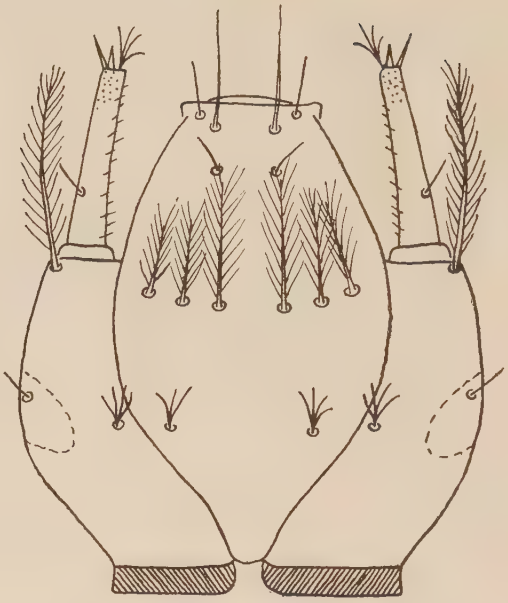


PLATE I

Morphology of adult *Anopheles koliensis*

TYPES.—Holotype female, allotype male reared in laboratory, Koli Area, Guadalcanal Island, August 6, 1944 (Owen). Paratype series A: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 14, 1944 (Owen), and 6 females, 6 males reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 6, 1944 (Owen). Paratype series B: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 14, 1944 (Owen), and 6 females, 4 males reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 4, 1944 (Owen). Paratype series C: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 14, 1944 (Owen), and 8 females, 3 males reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 6, 1944 (Owen). Paratype





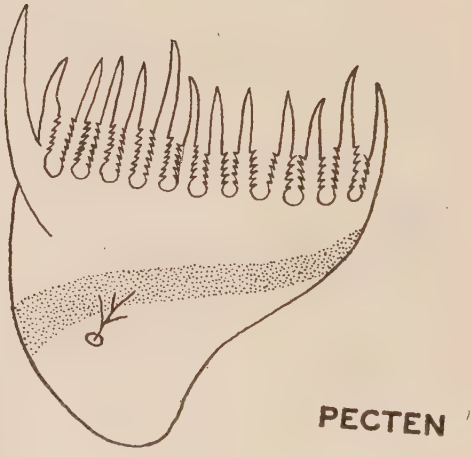


PLATE II
Morphology of larva of *Anopheles koliensis*

series D: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 14, 1944 (Owen), and 6 females, 5 males reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 6, 1944 (Owen). Paratype series E: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 14. 1944 (Owen), and 7 females, 5 males reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 7, 1944 (Owen). Paratype series F: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 14, 1944 (Owen), and 6 females, 4 males reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 6. 1944 (Owen). Paratype series G: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 14, 1944 (Owen), and 4 females, 4 males reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 6, 1944 (Owen). Paratype series H: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 14, 1944 (Owen), and 4 females reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 10, 1944 (Owen). Paratype series I: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 14, 1944 (Owen), and 9 females, 3 males reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 4, 1944 (Owen). Paratype series J: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 16, 1944 (Owen), and 5 females, 1 male reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 10, 1944 (Owen). Paratypes, 10 females collected resting in tent, Nalimbu River, Guadalcanal Island, July 13, 1944 (Owen). Paratypes, 4 females, 1 male reared from larvae collected in temporary pool at Metapona River Bridge, Guadalcanal Island, June 20, 1944 (Owen). Holotype, allotype and paratype series A, B, C, D, E, and F to be deposited in U. S. National Museum.

Identification.—This species is closely related to *Anopheles punctulatus* Dönitz and *Anopheles farauti* Laveran. Both males and females of *koliensis* can usually be separated from *punctulatus* by the absence of a small dark spot on the costal margin of the wing between the basal and median dark spots. The presence of a light patch of scales on the ventral side of the apical third of the labium in the female *koliensis* distinguishes it from the female of the above form. The larvae of this species are separated from *punctulatus* by having relatively short occipitals with three to four branches, enlarged shaft of prothoracic hair 1, well-developed palmate hairs on II, lateral hairs on IV bifurcate, and the structure of the pecten. As a rule the costal marking of the wing will separate both males and females of *koliensis* from *farauti*. The females of these two forms are also easily distinguished by the absence of light scales on the labium of *farauti*. The larvae of *koliensis* can be separated from *farauti* by having unbranched clypeal hairs, the basal tubercles of prothoracic hairs 1 and 2 separated, and the structure of the pecten. The species *koliensis* is closely related to another member of the *punctulatus* group which has a pale area on the ventral surface of the labium. This form is not known to occur east of New Guinea. It is usually classified as *Anopheles moluccensis,* Swg. and Swg. Whether these two forms are identical remains to be determined by future studies. The taxonomic relationships of the *punctulatus* group are obscure at the present. Rather than risk confusion by assigning the new material from the central Solomon Islands to *moluccensis* it was believed desirable to establish a new species.

Since *Anopheles koliensis* is morphologically in an intermediate position between *Anopheles punctulatus* and *Anopheles farauti,* the possibility that it is a hybrid of the two must be considered. Data which discredit this proposal are presented in a later section on experimental studies.

Distribution.—*Anopheles koliensis* is known to occur along the north coast of Guadalcanal Island from Koli Point to the vicinity of Aola Bay.

Biology.—The larvae of this species have been collected from temporary pools in the grassland and in the pools along the edge of the jungle. They prefer water exposed to sunlight rather than dense jungle conditions. They have always been associated with *farauti* and in one locality were collected from the same waters with

farauti and *punctulatus*. Information obtained on breeding habits of this species indicates that the larvae are relatively scarce during the dry season. No data have been obtained on breeding during the rainy season. The adults are strongly anthropophilic and are to be found resting during the day in native huts in greater numbers than any of the other local anophelines. Intensive studies were made in one locality for a period of several weeks on the relative abundance of adult anophelines found resting in tents occupied by personnel o fa native labor group. Three anophelines were encountered in these observations, namely *koliensis, farauti,* and *punctulatus*. Five hundred adult females collected from these tents were checked for identity and of this number 90% were *koliensis,* 5.8% were *farauti* and 4.2% were *punctulatus*. Data on relative abundance of the larvae of these three forms in this area during the period of the studies show that the larvae of *koliensis* constituted approximately 10% of the total anophelines. The results of this study emphasize the fact that *koliensis* has a greater preference for entering human dwellings than the other anophelines commonly found in the area. Limited observations on flight habits of the females have shown that they become active about 9:00 PM and continue to fly until daylight. The period of greatest nocturnal activity was after midnight.

Experimental Studies.—The species has been repeatedly reared in the laboratory by obtaining eggs from gravid females captured in native dwellings. The average time required to develop from egg to imago under these conditions is eleven days. Adult females reared in this manner and confined in a cage were observed to feed readily on man.

The two cardinal characteristics which separate the adults of *koliensis* from *farauti* and *punctulatus,* namely, the costal markings of the wing and the light patch of scales on the ventral side of the labium, were studied for degree of variation. The individuals utilized in this study were the progeny of fifty wild females of *koliensis*. Each of these gravid females was isolated and her progeny reared separately. The parents were kept for comparison with their offspring. A total of five hundred progeny were obtained and of this number three hundred were females and two hundred were males. A summary of this experiment is as follows: (1) Each parent had the typical wing marking of *koliensis*. (2) The size of the labial light patch varied among the parents from a small group of scales to a larger patch which was visible from above at the apex. (3) Among the female offspring there were only three that possessed evidence of a black spot on the wing between the basal and median dark spots. This character appeared in the wing on only one side of the body. (4) There were twelve of the male offspring which had a small dark spot between the basal and median dark spots of the wing. As was observed among the females, this character appeared in only one of the wings. (5) The size of the labial patch among the three hundred females exhibited no greater range in variation than was observed among the parents. There was also no pronounced correlation between the size of this character in the parent and her respective offspring. (6) The results of this study combined with an examination of several hundred additional specimens of this form leads to the conclusion that these two adult characters are quite stable.

Summary.—A new anopheline was discovered on Guadalcanal Island and is described as *Anopheles koliensis*. Evidence derived from breeding experiments of a limited nature and from an examination of several hundred wild adults establish it as a true species. Studies on habits of adults lead to the conclusion that it is of primary importance in disease transmission.

ANOPHELINE MOSQUITOES OF THE SOLOMON ISLANDS AND NEW HEBRIDES

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INTRODUCTION

Until less than three years ago, only two anopheline mosquitoes, *Anopheles punctulatus* and *A. punctulatus moluccensis,* had been reported as occurring in the Solomon Islands and New Hebrides. However, the presence of *A. punctulatus* was questionable as a state of confusion existed in regard to the exact taxonomic position of these species. Extensive collections during the past two years have revealed the existence of at least six species in this area. We feel that sufficient data have been accumulated to warrant a summary of the taxonomic status and the biology of these mosquitoes. Inasmuch as these species differ considerably in their habits, the presentation of this information should facilitate the determination of the relative importance of the disease transmitters as well as the formulation of control measures directed against the dangerous species.

The most intensive collections have been made on Espiritu Santo in the New Hebrides and on Guadalcanal in the lower Solomon Islands, and involved not only the rearing of adults from individual larvae taken from as many natural breeding places as possible, but also the rearing of progeny of isolated females, which had been captured in natural resting places and native dwellings. Such rearings enabled us to determine the extent of normal variation within "families," and to select the characters which remained constant and hence were of specific value. The distribution of the species in other islands of these groups is based upon material taken by a number of collectors.

KEY TO SPECIES

Adult Females

1. Costa without contrasting dark and light spots, palpi approximately one-third the length of the proboscis. ..... *Bironella hollandi*
   Costa with contrasting dark and light spots, palpi as long as the proboscis 2
2. Scutum with a vestiture of golden hairs, scales absent except on anterior promontory and near wing root; halteres white. 3
   Scutum with a vestiture of yellowish-white, broad, recumbent scales throughout; halteres dark-scaled on apex. 4
3. Apical third of labium yellow-scaled; fourth (morphological) segment of palpus with the basal dark ring covering approximately one-fifth of the segment *Anopheles lungae*
   Labium dark except for a small patch of bronzy yellow scales on the apical fifth or less; fourth segment of palpus with the basal dark ring covering approximately one-third of the segment. ..... *A. solomonis*
4. Labium dark-scaled except for an inconspicuous pale ring at extreme apex, rarely with a few ventral pale scales on basal half. ..... *A. farauti*

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Pale scales present on apical third to half of labium. 5

5. Apical third to half of proboscis entirely pale except for a narrow subapical dark ring and scattered dark scales ventrally, subapical ring occasionally absent; usually a small dark sectoral spot on costa between the basal and median dark spots. ..... *A. punctulatus*

Apical third of proboscis with a ventral patch of pale scales which may be limited to a few scales or may be extended dorsally near apex to form an incomplete ring; usually no dark sectoral spot on costa between basal and median dark spots. ..... *A. koliensis*

Adult Males

The characters used to separate the males of *Anopheles* are not entirely satisfactory; nevertheless, they will serve to distinguish the majority of specimens.

1. Costa without contrasting dark and light spots, palpi one-sixth of proboscis *Bironella hollandi*

Costa with contrasting dark and light spots, palpi as long as proboscis. 2

2. Scutum with a vestiture of golden hairs; scales absent except on anterior promontory and near wing root; halteres white. 3

Scutum with a vestiture of yellowish-white, broad, recumbent scales throughout; halteres black-scaled on apex. 4

3. Labium all dark-scaled except for apical light ring. ..... *Anopheles lungae*

Apical sixth to fifth of labium with a dorsal patch or incomplete ring of bronzy yellowish scales. ..... *A. solomonis*

4. A small dark sectoral spot on costa between basal and median dark spots. 5

No dark sectoral spot on costa between basal and medium dark spots. *A koliensis*

5. Labium with at least two or three ventral light spots, frequently large patches of light scales. ..... *A. punctulatus*

Labium all dark except for apical light ring. ..... *A. farauti*

Pupae

1. Paddle with fringe hairs on external margin only (Fig. 19); hair 5 of abdominal segment VI external to hair 10 on the posterior margin of the segment (Fig. 16); no oblique lateral margin to abdominal sternites. *Bironella hollandi*

Paddle with at least some fringe hairs internal to the paddle hairs (Fig. 20); hair 5 of abdominal segment VI internal to hair 10 on the posterior margin of the segment; sternites of abdominal segments with distinct oblique lateral margins. 2

2. Paddle with a series of strong denticles on the external margin (Fig. 20). 3

Paddle without denticles, fringe consisting of fine hairs only (Fig. 19). 4

3. Lateral spine (hair 1) of abdominal segment V approximately equal in length to that of segment III; lateral spine of segment VII extremely slender and pale, usually branched or apically frayed (Fig. 26, B and C). *Anopheles lungae*

Lateral spine of abdominal segment V approximately two to three times the length of that of segment III; lateral spine of segment VII stouter, darker, unbranched (Fig. 26, A). ..... *A. solomonis*

4. Trumpet pale, at most contrasting but little with the cephalothorax; hair 8 of the cephalothorax over 105 microns in length; hair 10 of abdominal segment II with 12 or more branches; lateral spine of segment VII elongate, slender, rarely showing any fraying (Fig. 26, H). ..... *A. punctulatus*

Trumpet brightly pigmented from apex to basal notch; hair 8 of cephalothorax under 100 microns in length; hair 10 of abdominal segment II with fewer than 12 branches; lateral spine of segment VII, shorter, stout. 5

5. Lateral spine of abdominal segment VII nearly always frayed or branched, shaped as shown in Fig. 26, D and E; apex of male genitalia as in Fig. 17. ..... *A. farauti*

Lateral spine of abdominal segment VII rarely branched or frayed (Fig. 26, G, shows the type of branching which occurs occasionally), shaped as illustrated in Fig. 26, F; apex of male genitalia as shown in Fig. 18.

A. koliensis

Fourth Instar Larvae

1. Hair 1 of mesothorax with stout shaft and many lateral branches; inner clypeals widely spaced, closer to outer clypeals than to each other; antennal hair minute. 2

Hair 1 of mesothorax with the structure of a small palmate tuft; inner clypeals closely approximated, with contiguous tubercles; antennal hair large, plumose. ..... *Bironella hollandi*

2. Prothoracic pleural group with one long hair branched; outer clypeals short, scarcely projecting beyond the clypeus or, if longer, then posterior clypeals 2–5-branched. 3

Prothoracic pleural group with all long hairs single; outer clypeals extending at least half their length beyond the clypeus, posterior clypeals single, rarely double. 4

3. Outer clypeals extremely short, scarcely projecting beyond the clypeus; posterior clypeals extremely short, not reaching the tubercles of the anterior clypeals, usually single, rarely double; hair 1 of abdominal segment II with long, slender, unnotched leaflets; long metathoracic pleural hairs usually single, rarely one split into 2 branches. ..... *Anopheles lungae*

Outer clypeals a third to a half as long as the inner; posterior clypeals extending to or beyond the tubercles of the anterior clypeals, with 2–5 branches; hair 1 of abdominal segment II almost as large as those of segment III, with broad notched leaflets; metathoracic pleural group with one of the long hairs usually split into 2–3 branches. ..... *A. solomonis*

4. Hair 1 of abdominal segment I a true palmate tuft, with broad, flattened leaflets; tubercles of prothoracic hairs 1 and 2 fused. ..... *A. farauti*

Hair 1 of abdominal segment I not resembling a palmate hair, but with narrow, hair-like branches; tubercles of prothoracic hairs 1 and 2 separate. 5

5. Outer occipitals usually with five or more branches (occasionally three to four); posterior clypeals short, not reaching the tubercles of the anterior clypeals; hair 2 of abdominal segments IV and V usually single or double; hair 6 of these segments usually double. ..... *A. koliensis*

Outer occipitals usually with 1–3 branches (rarely four); posterior clypeals usually extending to, or beyond, the bases of the anterior clypeals; hair 2

of abdominal segments IV and V usually triple; hair 6 of these segments usually triple. ..... *A. punctulatus*

CONSIDERATION OF SPECIES

Bironella (Brugella) hollandi Taylor

1934. *Bironella (Brugella) hollandi,* Taylor, Proc. Linn. Soc. N. S. W. **59:** 229 (♂ and ♀). **Type locality:** Kavieng, New Ireland. **Types:** Univ. of Sydney, Australia.

1944. *Bironella (walchi?).* Belkin and Schlosser, Jour. Wash. Acad. Sci. **34:** 268–273.

Adult Female.—A small, dark anopheline with unspotted wings and tarsi; palpi short. Length of wing 3–3.5 mm.

Head: White frontal tuft shorter than normal, lower setae barely reaching clypeus; vertical setae and long, erect, narrow, curved vertical scales white; erect occipital scales black, deeply forked and exceptionally long and slender, almost as long as frontal tuft setae. Antennae approximately two-thirds as long as proboscis; segments black except at base; torus dark brown; no scales on torus or first flagellar segment. Palpi usually slightly more than one-quarter length of proboscis, black-scaled. Labium black-scaled, slightly shorter than front femur. Labella light to dark brown. Buccopharynx without teeth.

Thorax: Scutal integument chocolate-brown; fossae, posterior extensions, and scutellum lighter; under proper illumination these areas, together with prescutellar space, and narrow median longitudinal scutal line, gray pollinose; scutellar disc usually dark, or entire scutellum often dark; scales entirely absent; very sparse vestiture of golden hairs; bristles dark. Halteres light basally, upper two-thirds of stem and knob dark; knob and part of stem with very short dark scales. Pleural integument variable in color, usually dark with lower mesepimera and lower sternopleura pale; scales entirely absent. Propleurals absent; spiraculars absent; lower sternopleurals 4–6 light hairs; upper sternopleurals 2 long dark bristles; prealars 3–5 light bristles; lower mesepimerals absent; subalar 1 dark bristle.

Wing: Vein scales, except on costal border, light gray, appearing white under proper illumination; fringe a slightly darker shade of gray; entire length of costa and vein 1 to slightly beyond middle of wing with scales much darker and denser giving appearance of black costal border in sharp contrast to rest of wing which appears light gray. Vein 2 approximately one and one-quarter times length of vein 2.1; vein 3 extending basad from cross-veins as a line of scales usually one-third of distance to wing base, just distad of cross-vein, usually a slight upward curvature; petiole of second posterior cell (vein 4 distad of cross-veins) approximately as long as vein 4.2, a downward curvature near its middle; vein 5.1 with downward curvature just distad of cross-vein; vein 6 with slight curvature in middle and at base; base of second posterior cell distinctly basad of second marginal cell; cross-vein at base of vein 2 clouded; cross-veins 2–3 and 3–4 in line; cross-vein 4–5 usually just basad of 2–3 and 3–4 and parallel to them.

Legs: Front femora slightly swollen, dark except for narrow central pale line on posterior surface from near base to apex, connected at base with a dorsal pale line which extends dorsally for approximately half the length of femur, anterior surface with a few scattered pale scales; front tibiae dark except for a narrow line of pale scales ventrally on posterior surface. Middle femora dark except for narrow pale line from base to within tenth of apex; middle tibiae dark except for narrow pale line from base to apex. Hind femora with entire anterior surface pale-scaled from base to apical fifth, posterior surface mottled with pale scales from base to apical fifth, narrow line of pale scales dorso-posteriorly extending halfway to apex; hind tibiae with narrow line of pale scales on anterior surface from basal sixth to apical fifth, posterior surface dark. Coxae and trochanters pale. All tarsi dark dorsally, lighter ventrally. Claws small, simple, and equal on all legs.

Abdomen: Dark brown, covered with moderately dense brown hairs on sternites and tergites, hairs more numerous and lighter on distal segments; no scales; cerci without scales, densely covered with light hairs.

Adult Male.—In general as in the female. White scaling on head more restricted; occipital scales fewer in number. Palpi usually slightly less than one-sixth the length of proboscis. Proboscis almost one-fifth longer than front femur. Thoracic vestiture even more restricted. Fifth segment of front tarsus shorter than fourth, cylindrical; claw simple. Sidepieces with very narrow dark scales.

Genitalia (Figs. 3 and 4): Sidepiece short, stout, about twice as long as broad; outer surface clothed with rather short hairs; a patch of long, narrow scales basally on dorsal surface. Basal lobe prominent, with a group of 9–15 stout spines at apex, 8 spines according to Taylor. A dense clump of long hairs arising proximally from ventral surface of basal lobe. Clasper long,

slender, bulbous at base and narrowing abruptly distally, sharply curved before apex; terminal spine short, rounded, and only slightly projecting beyond tip of clasper. Mesosome very long, slender; at the apex a pair of long, downwardly and laterally curving forked appendages, the branches of which are coarsely serrate. Claspette long, erect, the dorsal surface strongly convex medially, this convexity clothed with a dense patch of short, appressed, curved spines; apex with a stout digit-like process and two membranous appendages with expanded tips.

PUPA.—*Cephalothorax:* Trumpet (Fig. 23) darkly pigmented, scoop-shaped. Head shield distinctively shaped (Fig. 27), index of width to length 1 : 1.16 (range 1.00 to 1.35). External margin of palpal case evenly rounded (Fig. 27). Internal margin of median keel indistinct. Metanotal bar with a series of longitudinal wrinkles of which the median is heaviest, no definite lateral margins (Fig. 16).

Abdomen (Fig. 16): Hair 5 of segment VI external to hair 10.[1] Lateral spine (hair 1) of segment V five to ten times the length of that on III, generally twice the length of that of IV, and one-half to three-fourths the length of the lateral spines of VI and VII; lateral spines of segments IV–VII gradually tapered, with rounded apices, never branched or frayed (Fig. 25). Table 3 summarizes the number of branches possessed by the more important hairs.

Paddle (Fig. 19): Buttress only slightly developed. External border of midrib distinct to posterior one-fourth, internal border indistinct; no granulation of midrib or buttress. Fringe on external margin only, extending from upper one-fourth posteriorly to hair 12, consisting of long fine hairs. Hair 12 short, stout, straight, dark and sharply tapered. Hair 13 fine, approximately equal in length to 12, and pale.

Genitalia: Figs. 22 and 24 illustrate the male and female pupal genitalia.

LARVA.—*Head* (Fig. 8): Inner anterior clypeals long, single, closely approximated, the tubercles contiguous; outer clypeals single, one-third to one-half the length of the inner; posterior clypeals very small, single or double. Frontal and subantennal hairs long, plumose. Occipital hairs short; inner 2–4-, outer 2–5-branched. Terminal hair of antenna long, plumose. Antennal hair 5–13-branched, situated one-third from base and reaching almost to or beyond apex of antenna.

Thorax: Prothoracic hair 1 without tubercle; stem short, slender, with 8–16 long, radiating branches; hair 2 with small tubercle, about twice the length of hair 1, with 12–15 branches (Fig. 9). Prothoracic pleural hair 9 long, split distally into 2–4 branches; hair 10 long, single; hair 11 small, 2–4-branched; hair 12 long, single (Fig. 10). Anterior mesothoracic pleural hairs (Fig. 11) long; hair 11 very small, single; hair 12 single, about one-fourth to one-third the length of the anterior pair. Anterior metathoracic pleural hairs (Fig. 12) long, single; hair 11 very small, single; hair 12 short, 2–4 branched. Hair 1 of meso- and metathorax with the structure of a small palmate tuft.

Abdomen: Palmate hairs present on segments I–VII; those on segment I somewhat reduced; leaflets long, narrow, edges smooth. Anterior tergal plates large, in width about two-thirds the distance between the palmate hairs; dorsum of segment VII almost entirely covered by tergal plate. Small posterior tergal plate present on segments III–VII. Hairs 6 and 7 of segments I and II long, plumose; hair 6 of segment III long, but with fewer branches; hair 6 of segments IV and V long, single. Postspiracular hair long, with about 5 branches; saddle hair long, single. Pecten with 6–8 short and 6–9 long spines.

EGG (Fig. 7). Broad anteriorly, tapering posteriorly; color black; approximately 0.55 mm. in length by 0.2 mm. in width (including floats). Floats large, almost as long as the egg; 15–20 float ridges. Frill with the shape of a broad collar at anterior end. Exochorion with fine reticulations forming an irregular, polygonal pattern.

Taxonomic Discussion.—The subgenus *Brugella* to which this species is assigned is characterized by short palpi, simple antennae in the male as well as the female, and by the absence of the basal arm and the presence of a clump of stout spines at the base of the sidepiece in the male genitalia. *B. hollandi* Taylor and *B. travestitus* (Brug) are the only species of the subgenus known in the adult stage at the present time. The original and subsequent descriptions of *B. travestitus* are too brief in their treatment of adult morphology and ornamentation to permit the separation of the two species in the female. The characters of the darker pleurae

[1] The position of hair 5 of abdominal segment VI in *Bironella* seems to offer excellent evidence that hair 9 of the genus *Anopheles* is actually hair 5, but shifted just internal to 10. Because of this, the nomenclature system for pupal hairs proposed by Rozeboom and Knight (1945, m. s.) and utilized here is being modified by changing hair 9 of segment VI to hair 5.

and differences in the curvature of veins used by Taylor to distinguish *B. hollandi* from *travestitus* are variable in the former species. *B. hollandi* and *travestitus* can be differentiated by the details in the structure of the claspette, and by the prominent basal lobe with its patch of long hairs and 8–15 stout apical spines in the former species. In *travestitus* there are only 4 long spines at the base of the sidepiece, and they apparently are not situated on a prominent lobe; furthermore, the group of long hairs seems to be absent (Swellengrebel and Rodenwaldt, 1932).

The larvae of *B. hollandi, travestitus, gracilis,* and *walchi* are characterized by the large plumose antennal hair and by the long external clypeals, which are at least a third as long as the inner clypeals. The known larvae of the other species of *Bironella* have a small antennal hair and very short outer clypeals, which are less than a fourth the length of the inner clypeals. Van Hell (1938) describes the long pleural hairs of *travestitus, gracilis,* and *walchi* as being single; in *hollandi* hair 9 of the prothoracic pleural group is split into 2–4 branches (Fig. 10). The outer clypeals of *walchi* are as long as the inner, while in *hollandi* they are one-third to one-half the inner. Prothoracic hair 1 of *walchi* is only 4–5-branched; in *hollandi* it is 8–16-branched. Taylor suggests the possibility that *hollandi* is the same as *walchi,* but this is not supported by the differences between the larvae. In *gracilis* the outer clypeals are 2–5-branched or even plumose; in *hollandi* they are single. The larva of *travestitus* seems to be very similar to that of *hollandi,* except for the outer anterior pleural hair (hair 9).

Distribution.—Guadalcanal. Northwest and north central coast: Marovovo, Visale, Tenamba, Aruligo, Doma Cove, Segilau, Bunina, Tassafaronga, Bonegi, Mamara, Poha, Kokumbona, Matanikau, Kukum, Lunga, Teneru, Koli; southwest coast: West Cape. Russell Islands. Banika (W. G. Downs). New Georgia Group. Munda (J. G. Franclemont). Bougainville. Empress Augusta Bay (A. B. Gurney).

Biology.—On the northwest coast of Guadalcanal *Bironella hollandi* is the commonest anopheline breeding in undisturbed, natural, permanent or semi-permanent bodies of water, such as streams, swamps, and dense coastal lagoons at the mouths of rivers. Bomb craters, "fox holes," road ruts, hog wallows, and even artificial containers in at least partial shade are sometimes utilized as breeding places. The larvae occur most frequently in shaded situations, and have been taken in places so dark that they could not be seen in a white dipper. Although they have been collected in very exposed situations, such as small canebrake swamps in grasslands, even here they are usually found deep in the vegetation where there is very little light. The dry season of the year forces the species into quite open situations such as pools in beds of dry streams. During the rainy season, the streams are flushed, the swamps expand and larvae are more difficult to find, although the species continues to breed in large numbers, as collections through every month of the year indicate. The larvae tolerate water with a high organic content such as is found in dense canebrake swamps, but occur more frequently in cool, clear water in vegetation or flotage.

The anophelines most frequently associated with this species are *A. lungae* and *solomonis.* In more exposed situations it is frequently associated with *farauti* and very rarely with *punctulatus.* A species of *Dixa,* several species of *Culex* (*Lophoceratomyia*), *Uranotaenia argyrotarsis* Leic. and *U. tibialis* Taylor are frequently found with *B. hollandi.*

There are two color phases of the larvae of this species, light yellowish-brown and black. Both phases may be recognized macroscopically from other local anophelines by the light coloration of the entire fourth abdominal segment which is in strong contrast with the uniformly darker coloration of the rest of the abdomen; the other anophelines may have light markings on the abdomen, but these are not restricted to the fourth segment. The pupae are smaller and wider than those of the other anophelines and have shorter and more widely-flared trumpets. The eggs float on the surface of the water like those of other anophelines, but can be distinguished from local species by the meniscus they form on the surface, which is straight instead of being cross-shaped.

Larvae brought from the field are rather difficult to rear and take approximately ten days to two weeks from the first instar to pupation; the pupal stage lasts two to three days. The adults are very fragile and are difficult to keep alive in captivity. They exhibit the normal anopheline resting position, but because of their extremely thin bodies and excessively elongate legs, they resemble small crane flies. Nothing is known about their habits beyond the fact that the females do not feed on man. All the adults in collections have been reared. Searches for resting places have been made without success in the vicinity of breeding places.

Anopheles (*Myzomyia*) *solomonis*, n. sp.

Adult Female.—A medium-sized, yellowish-gray, speckled anopheline; apical sixth to fifth of labium with dorsal patch or ring of yellow scales. Length of wing, 4 mm.

Head: Conspicuous white frontal tuft; vertical setae white, followed by one or two rows of white narrow hair-like scales; white scales in middle of vertex forming a wide spot narrowed in center; posteriorly the light scales with a yellow tinge; the rest of the vertical and all occipital scales dark. Antennae with a few minute white scales on torus, dense white scaling on first flagellar segment. Palpi as long as proboscis, ornamented as shown in Fig. 2; second morphological segment black with very narrow ring of white scales; third segment black with very narrow incomplete ring of white scales; fourth segment black-scaled basally for a third of its length, remainder white in center and yellowish basally and apically; fifth segment black-scaled for a third of its length, remainder yellowish. Ratio of fourth segment to third segment 1 : 1.7. Labium (Fig. 2) dark-scaled except for a dorsal patch of yellowish bronzy scales which may extend ventrally, and an apical light ring; differentiation between the coloration not distinct. Labella dull yellow. Buccopharyngeal armature (Fig. 6) of several broad central teeth forming a single row; teeth of similar character, separated by intervals; apices of teeth deeply serrated, bases without bullae or lateral spines.

Thorax: Scutal integument light yellow-brown with gray pollinose longitudinal lines; dark brown eye spots in front of and behind scutal angle; prescutellar space and disc of scutellum dark brown. White scales on anterior promontory rather short and sparse, central scales elongate, lateral broader; 15–25 black scales on humeral angles below lateral tufts. Rest of scutum devoid of scales; vestiture consisting of numerous golden hairs of varying length. Scutal and scutellar bristles light in color. Prothoracic lobes with a large patch of short black scales on upper part. Pleura without scales, integument dark gray with usual darker lines and spots. Spiracular bristles absent; propleurals 6 stout dark bristles; lower sternopleurals several golden hairs; upper sternopleurals 2–3 dark bristles and varying number of light hairs; prealars 4–8 golden hairs; subalars 5–10 golden hairs; lower mesepimerals absent. Halteres white with white scales on shaft and knob.

Wing (Fig. 1) : On the costal border three dark and three light humeral spots, third light humeral spot sometimes obliterated; basal dark spot solid on costa, subcosta and vein 1, usually narrowed on 1; sectoral spot variable in size; accessory sectoral spot usually reaching costa; dark sectoral spot narrowed on subcosta, frequently absent on vein 1; median dark spot very large, wedge-shaped, extending to base of vein 2, on vein 1 dark scaling may be interrupted by light scales distally, small dark spot thus formed migrating occasionally into subcostal spot; subcostal spot large; preapical dark spot extremely variable, may be large and solid on both costa and vein 1 or on costa alone, or broken into two to four small spots on these veins, or represented by one very small spot on each one of these veins; preapical spot sometimes reduced;

apical dark spot solid, including veins 1 and 2.1; apical spot smaller than apical dark spot. Wing veins light-scaled with the following dark spots: vein 2 with large dark spot involved in median dark spot and one to two small dark spots distad; vein 2.1 with 2 to 3 basal small dark spots and one large spot involved in apical dark spot, apex of vein light-scaled; vein 2.2 with 5 to 7 small dark spots, evenly spaced, vein dark at apex; vein 3 with 5–8 small dark spots, apex of vein light; vein 4 with 3 to 7 dark spots, large one usually at bifurcation; veins 4.1 and 4.2 each with 2 to 4 dark spots, large one usually at base continuous with apical dark spot of vein 4; vein 5 with 3–6 rather evenly spaced small dark spots; vein 5.1 and 5.2 each with 3–5 small dark spots; vein 6 with 4 to 7 small dark spots. Fringe white except for the following dark spots: large spot between veins 2.1 and 2.2; small spots below apex of veins 3 (frequently absent), 4.1, 4.2, and 5.1; large spot at apex of vein 6. Light wing scales yellow, dark scales black. Venation and cross-veins normal.

Legs: Front femora swollen in basal half, speckled with light scales; middle and hind femora and all tibiae with numerous, rather evenly spaced pale spots. First segment of all tarsi with numerous light spots and light apex. Second, third, and fourth segments of front tarsi with broad basal and apical light bands; second and third segments often with additional light spots in center; fifth segment dark except at apex. Second, third, and fourth segments of middle tarsi with narrow apical light bands, occasionally with light spots centrally; fifth segment dark except at extreme apex. Second segment of hind tarsi with narrow apical light band and usually one to three small light spots centrally; third segment with narrow apical light band and occasionally a few light scales centrally; fourth segment with narrow apical light band; fifth segment all dark. A few light scales basally on middle and hind tarsal segments, visible under high magnification. Extent of light scaling on legs very variable. Light scales on legs yellowish.

Abdomen: Integument dark gray. Devoid of scales on tergites and sternites I–VII, instead vestiture of narrow golden hairs similar to those on scutum. Hairs more numerous on posterior segments, grading into very narrow curved scales on tergite and sternite VIII. Cerci with narrow black scales.

Adult Male.—In general as in the female. First flagellar segment with tuft of narrow white scales. Second palpal segment with light scales dorsally in center; articulation between second and third segments light-colored, third palpal segment with narrow apical ring of yellow scales and patch of yellow scales dorsally in center; fourth and fifth segments yellow with narrow basal dark rings which extend externally to near apex of segments. Labium dark except for dorsal patch on incomplete ring of bronzy yellowish scales on apical sixth to fifth. Abdomen as in female except for numerous yellow scales on eighth tergite. Sidepieces densely covered with yellow scales ventrally (morphologically) and black scales laterally and dorsally.

Genitalia: Indistinguishable from those of *A. lungae.*

Pupa. The pupa possesses a brownish-yellow color which persists to a distinctive degree even in specimens reared completely through in the laboratory. This is in marked contrast to the *punctulatus* series, the members of which become nearly devoid of pigment in material reared from eggs. Tables 2 and 3 summarize the data of diagnostic value for this species.

Cephalothorax: Trumpet dark brownish-yellow. Index of width to length of head shield 1 : 0.98 (range 0.93–1.06). External margin of palpal case rounded, sometimes with an angulate point at the center of the curve. Internal margin of median keel sharply outlined. Metanotum with a distinct, smooth median bar bounded laterally by a crease which is incomplete posteriorly. Chaetotaxy similar to that of *B. hollandi* except that hair 11 is generally slightly anterior to a line drawn between 10 and 12.

Abdomen: Chaetotaxy generally similar to that shown for *B. hollandi* (Fig. 16) except for the following: sternites with strong oblique lateral margins. On segment I, hair 6 generally external to hair 9, occasionally in line with it; on segment III, hair 6 well anterior to a transverse line through hair 4, hair 18 absent; on segment IV, hair 6 well anterior to a transverse line through hair 5, on segment V, hair 5 nearer to hair 4 than to 8, much shorter than 8 in length; on segment VI, hair 2 on or very near the posterior margin of the segment, hair 5 on the posterior margin internal to 10, shorter than 10; on segment VII, hair 2 on the posterior margin of the segment, hair 5 well anterior to a transverse line through hair 4; on segment VIII, hair 8 very near to a transverse line through the lateral spine (hair 1). Lateral spine of segment V approximately two to three times the length of that of segment III; lateral spines of segments VI–VII (Fig. 26, A) slender, elongate, acutely tapered and only very rarely with branches or fraying, pigmented to about the same extent as the segments.

Paddle (Fig. 20): External border with stout, bluntly tapered denticles, which are pigmented to the same degree as the paddles. These denticles begin at upper one-fourth as minute teeth internal to the border, increasing in size posteriorly and extending to and along the external margin to the postero-lateral corner, the last few denticles being more acutely pointed

than the others; a fringe of long, pale hairs begins where the denticles terminate and extends mesally and forward along the inner margin of the paddle to the anterior one-fourth. External border of midrib obscure, internal border absent or very faint, granulations scanty or absent. Buttress with minute, scattered denticles and generally with areas of granulations. Hair 12 slender, pale, straight or curved. This hair is much in contrast to that of the *punctulatus* series where it is noticeably stouter and longer.

Genitalia: In male (Fig. 17), more heavily pigmented than any other part of the abdomen; the lateral margins of the genital lobes are nearly parallel from base to apical one-fourth, apex of lobes shaped as figured. Female genitalia similar in form to that illustrated for *farauti* (Fig. 21).

LARVA.—*Head:* Inner clypeal hairs very long, with many minute lateral branches; outer clypeals a third to a half the length of the inner, sometimes single but usually frayed; the distance between the outer and inner hairs one-fourth to one-third that between the inner hairs; posterior clypeals 2–5-branched and long enough to reach the tubercles of the anterior clypeals. Frontal and subantennal hairs feathered. Antennae with long spines on inner surface; antennal hair minute, situated one-third the distance from the base; terminal hair longer than sabers, 4–8-branched. Occipital hairs short; inner 2–5-branched, outer 2–6-branched.

Thorax: Tubercles, of prothoracic hairs 1 and 2 large, heavy, separated from one another; hair 1 with heavy shaft and many long radiating branches; hair 2 about twice the length of hair 1. Prothoracic pleural hair 9 (Fig. 13) long, with 5–10 branches; hair 10 long, single; hair 11 about one-fourth the long hairs, single or double; hair 12 long, single. Anterior pair of mesothoracic pleural group long, single, occasionally one hair double; hair 11 minute, single; hair 12 short, 2–4-branched. Outer anterior metathoracic pleural hair (9) split into 2–3 branches, inner hair long, usually single, sometimes double; hair 11 minute; hair 12 short, 2–4-branched (Fig. 14).

Abdomen: Hair 1 on segment I very small, with narrow, hair-like branches. Hair 1 on segment II almost full-sized, with broad, notched leaflets. Palmate hairs large on segments III–VI, smaller on segment VII, heavily pigmented on segments II–VII; leaflets very broad, abruptly narrowed, with deep indentations, into a short terminal filament that is about one-fourth as long as the main shaft. Hair 6 of segments IV and V 2–4 branched. Tergal plates darkly pigmented, small, half or less the distance between the palmate hairs except on segment VIII where it is large. Hair 13 of Segment V very large, with 7–13 branches. Pecten with 4–5 long and 6–10 short spines. Saddle hair long, single.

TYPES.—**Holotype** ♀ with larval and pupil skins, Poha River tributary, 3 miles south of the coast, Guadalcanal, 10 Sept. 1944 (L. E. Rozeboom). **Allotype** ♂ with larval and pupal skins, Bonegi River tributary, 2½ miles south of the coast, elevation 1500 feet, Guadalcanal, 21 July 1944 (L. J. Lipovsky). **Paratypes** (29 ♂, 41 ♀): 1 ♂ with pupal skin, Matanikau River tributary, Guadalcanal, 27 Nov. 1943 (J. N. Belkin); 1 ♀ with larval and pupal skins, Matanikau River tributary, Guadalcanal, 8 Feb. 1944 (J. N. Belkin); 1 ♀, Burns Creek, Lunga District, Guadalcanal, 25 May 1944 (J. N. Belkin); 2 ♀, 1 ♂ with larval and pupal skins, Bonegi River tributary, 2½ miles south of the coast, elevation 1500 feet, Guadalcanal, 21 July 1944 (L. J. Lipovsky et al); 1 ♂ with larval and pupal skins, Matanikau River tributary, Guadalcanal, 6 Aug. 1944 (J. N. Belkin); 1 ♂, 2 ♀, Kokumbona River, 4 miles south of coast, Guadalcanal, 24 Aug. 1944 (M. Cohen, H. Sexauer, et al); 1 ♂, 2 ♀ with larval and pupal skins, 2 ♂, 3 ♀ reared from pupae, Poha River tributary, 3 miles south of coast, Guadalcanal, 9–26 Sept. 1944 (L. E. Rozeboom, J. N. Belkin, J. Laffoon, et al); 22 ♂, 30 ♀ with larval and pupal skins, progeny from gravid female collected resting on tree trunk, Sprague Swamp, Bunina, Guadalcanal, 13 Nov. 1944 (L. J. Lipovsky, M. Cohen, A. W. Barnes). **Holotype** and **allotype** to be deposited in U. S. National Museum. **Paratypes** to be deposited in the collections of the University of Sydney, Cornell University, Johns Hopkins School of Hygiene and Public Health.

Taxonomic Discussion. *A. solomonis* and *lungae* form a distinct complex and perhaps should be recognized as a separate series under the group *Neomyzomyia*. They fall into this group on the basis of buccopharyngeal armature (Figs. 5, 6), propleural hairs, presence of scaling on the pronotal lobes, and speckled legs in the adults. Other species of this group in the Oriental and Australasian regions having the scutal ornamentation restricted to the anterior promontory, entirely white halteres, speckled legs without a broad white band on the hind legs at the junction of the tibia and first tarsal segment are *A. tesselatus, longirostris, longirostris annulata*, and presumably *tesselatus orientalis*. *A. solomonis* and *lungae* differ from

all of these in having a very narrow ring of white scales at the apex of third palpal segment instead of a broad light band, covering usually about a half of this segment.

The larval pleural hairs show branching which is inconsistent with the definition of the group *Neomyzomyia,* but which is found in three other forms usually referred to this group: *A. amictus, amictus hilli,* and *novaguinensis.* The latter species all have one long hair of the mesopleural group at least two-branched, and one long hair of the metapleural group with five or more branches. In *A. solomonis* and *lungae,* the long mesopleurals are usually single, and both long metapleurals may be single (usually in *lungae,* rarely in *solomonis*), or at most with 2–3 branches (occasionally in *lungae,* usually in *solomonis*).

The adults of *A. novaguinensis, amictus* and *amictus hilli* possess conspicuous scales on the disc of the scutum which immediately separate them from *solomonis* and *lungae.*

Adult females of *A. solomonis* are separated from *lungae* by the restricted yellow coloration on the apical fifth or less of the labium, the proportionately shorter third palpal segment, the greater extent of the dark coloration on the base of this segment, the generally darker coloration of the legs, and differences in the buccopharyngeal armature. All males of *solomonis* examined showed some pale scales on the labium, a character which is very rarely present in *lungae.* The larvae of the two species are separated on the size of the outer clypeals, the size and branching of the posterior clypeals, and the structure of the palmate hair on abdominal segment II.

In a personal communication to one of the authors (JNB), J. G. Franclemont reports finding a form similar if not identical with *A. solomonis* on New Georgia Island. The only obvious difference noted in the brief description available is in the more extensive yellowish coloration of the proboscis which may cover the apical third.

Distribution.—Guadalcanal. Northwest coast: Bunina, Tassafaronga, Bonegi, Poha, Kokumbona, Matanikau, Lunga. ? New Georgia. Munda (J. G. Franclemont).

Biology.—The larvae of *A. solomonis* have been collected most frequently in small tributary streams of the larger rivers at a distance of one to four miles into the coral foothills of the northwest coast of Guadalcanal at elevations to 1500 feet. They were found in pot holes in coral stream beds, in coral depressions above the stream beds, along the margins of the streams, and in the blocked mouths of the tributary streams where they are probably flushed from breeding places farther up the streams. One collection was made in a taro swamp with a depth of water of only one inch. The water in the breeding places may be clear and running, but is frequently stagnant, with a high organic content, and bluish in color. Practically all the collections have been made in deeply shaded areas in flotage of sticks or in leaves along the margins of the pools. The larvae are difficult to locate and are very scarce. They have the same habits as *lungae* of crawling up on leaves and banks out of the water. The pupae also work their way out of the water, as do those of *lungae.* The species may be collected throughout the year but is less difficult to collect during the dry months.

A. solomonis is frequently found where other anophelines are absent, but it has also been collected in association with *A. lungae, B. hollandi,* and once with *A. farauti* and *punctulatus.*

The larvae and pupae resemble macroscopically those of *A. lungae* in the shape of the body and coloration and cannot be separated from this species in the field.

From laboratory rearings, the length of life cycle appears to be the same as for *A. lungae*. All the eggs do not hatch at once and the larval stage lasts from 10 days to 2 weeks or longer, and the pupal stage from two to three days.

Little is known concerning the habits of the adults. Both males and females have been found in very small numbers resting in the daytime in the company of *A. lungae* on the buttresses and trunks of large trees in swampy jungle areas.

Anopheles (*Myzomyia*) *lungae* Belkin and Schlosser

1944. *Anopheles* (*Myzomyia*) *lungae* Belkin and Schlosser, Jour. Wash. Acad. Sci. **34:** 268 (♂ and ♀). **Type locality:** Guadalcanal Island. **Types:** U. S. National Museum.
1944. *Anopheles* sp., Knight, Bohart and Bohart. Keys to the mosquitoes of the Australasian Region. Nat. Res. Council, Wash., p. 10.

Adult Female.—A medium-sized, yellowish, speckled anopheline; apical third of labium yellow-scaled. Length of wing 4–4.5 mm.

Palpi as long as proboscis; second and third morphological segments black with narrow ring of white scales apically, in 5 per cent of specimens studied (80), dorsocentral patch of dark bronzy scales also present on these segments; fourth segment black-scaled basally for approximately one-fifth its length, remainder white except frequently yellowish basally and apically; fifth segment black-scaled for approximately one-fifth of its length, remainder yellowish; ratio of fourth segment to third segment 1 : 1.35. Labium dark basally; apical third golden-yellow-scaled except usually a preapical dark ring; boundary between black and yellow sharply marked; in less than 5 per cent of specimens examined a few light scales basad of light apical area. Labella dull yellow. Buccopharyngeal armature (Fig. 5) of several teeth forming a single row; teeth of similar character, separated by intervals; apices of teeth serrated, bases with bullae but without lateral spines. Scutum without scales except on anterior promontory and in front of wing roots, vestiture of numerous golden hairs of varying length. Scutal integument light yellowish-brown; eye spots and dark areas as in *solomonis*. Prothoracic lobes with patch of black scales dorsally. Pleura without scales. Lower mesepimerals absent; propleurals 6 stout dark bristles; other pleural bristles and hairs as in *solomonis*. Halteres white with white scales on shaft and knob. Wing in general as described for *solomonis*. Legs as in *solomonis*, except that on front tarsi fifth segment usually with basal and apical light bands, fourth segment frequently with light spots in center dorsally; light coloration of front legs more extensive than in *solomonis*. Abdomen devoid of scales on tergites and sternites I–VII; vestiture of golden hairs of varying length; hairs more numerous on posterior segments, grading into narrow curved scales on apical border of segment VIII. Cerci with narrow yellow scales at apex, dark scales at base and sides.

Adult Male.—In general as in the female. Second morphological palpal segment usually with few white scales at apex and patch of yellow scales dorsocentrally; third segment with narrow apical yellow ring and patch of yellow scales dorsocentrally; fourth and fifth segments yellow with usually narrow basal dark rings; extent of light coloration variable. Abdomen and sidepieces as in *A. solomonis*. The male genitalia have been described and figured by Belkin and Schlosser. Some of the leaflets of the mesosome are serrate.

Pupa.—Very similar to the pupa of *A. solomonis* except for the following: lateral spines of abdominal segments IV–V each approximately equal in length to that of segment III; lateral spines of segments VI and VII (Fig. 26, B and C) extremely slender and pale, appearing to be less pigmented than the segments, frequently branched or apically frayed; the lateral spine of segment VII possessing one or more branches in over 80 per cent of the specimens examined. Tables 2 and 3 summarize the data of diagnostic value for the species.

Larva.—Inner anterior clypeal hairs widely spaced, rather short, frayed; outer clypeals very short, extending at most only slightly beyond the clypeus; posterior clypeals single, rarely double, and very short, not reaching the tubercles of the anterior clypeals. Inner and outer occipitals short, 2–4-branched. Tubercles of prothoracic hairs 1 and 2 heavy, separated from one another; hair 1 with stout shaft and long radiating branches. Outer anterior hair (9) of prothoracic pleural group (Fig. 15) split into 3–6 (rarely 2) branches; anterior hairs of meso- and metathoracic pleural groups long, single.

Hair 1 of abdominal segment I with narrow, leaf-like branches. Hair 1 of abdominal segment 2 with long slender, unnotched leaflets. Palmate hairs large on segments III–VI, smaller on segment VII. Hair 6 of segments IV and V sometimes 1–2-, usually 3-branched.

Distribution.—GUADALCANAL. Northwest and north coast: Tenamba, Aruligo, Doma Cove, Segilau, Bunina, Umasami, Tassafaronga, Bonegi, Mamara, Poha, Kokumbona, Matanikau, Kukum, Lunga, Tenaru, Koli, Tetere. NEW GEORGIA. Munda (J. G. Franclemont). BOUGAINVILLE. Empress Augusta Bay (A. B. Gurney).

Biology.—"The larvae of this species are normally found in the jungle in seepage areas, along the margins of streams, pot holes in stream beds, rock holes, dense jungle swamps, and temporary pools. The species has a decided preference for shade in its breeding places" (Belkin and Schlosser, 1944). Other breeding places noted are "fox holes," hog wallows, old steel helmets, dense canebrake swamps, and coastal lagoons at mouths of streams. The water in some breeding places may have a very high organic content, but usually is clear and cool. The larvae at times become quite numerous, but are hard to collect because of their habit of resting in very shallow water on the margins of the breeding places. They have been observed to crawl out of water and rest on the bank or on dead leaves floating ing on the water. The pupae also leave the water and in the laboratory normally rest on the sides of the rearing vessels, sometimes half an inch or more above the surface film. A film of water completely surrounds them, but does not connect with the surface of the water in the container. Usually emergence takes place from pupae resting above the surface of the water.

A. lungae increases in numbers during the rainy season when the breeding places become more extensive. The aquatic stages are even flushed from the hills into breeding areas in the coastal plain. On two occasions larvae and pupae were observed floating in a strong current in a small stream draining a large canebrake swamp. Several hundred larvae and pupae were collected in an hour's time among the debris held in place in the middle of the creek by an obstruction.

A. lungae has been collected commonly in association with *B. hollandi* and *A. solomonis,* rarely with *farauti,* and only once with *punctulatus.*

The larvae of *A. lungae* are easily distinguished in the field, except from *solomonis,* by the relatively broader abdominal segments, the transparency of the body and clear yellow pigmentation. The pale larvae of the other species have a much more opaque coloration (*A. punctulatus* and *farauti*) or are very narrow (*B. hollandi*).

A large number of rearings from eggs have shown some unusual characteristics of this species. All eggs do not hatch at once, even though they may be floating on the surface of the water. Some hatch three to four days after oviposition, others may not hatch for two weeks or longer. We have obtained adults from a batch of eggs before all first instar larvae appeared. The eggs are also capable of withstanding a considerable amount of drying. This seems to be an adaptation to breeding places which are periodically flushed. The larval development, once started, takes about ten days; the pupal stage usually lasts two to three days.

The diurnal resting places of the adults are easily located in swampy jungle areas where the species breeds in abundance. Males and unfed, blooded or gravid females are found together resting on the trunks and buttresses of various species of large trees growing in such areas. On several occasions they have been observed to rest also at the bases of various herbaceous plants. In uncontrolled areas, two or three hundred adults have been occasionally collected from a single tree. They

usually rest a foot or less above the ground where the humidity is very high and where there is very little light, but they have also been frequently collected resting as high as six feet above the ground on well-illuminated portions of the trunks. In some areas a third or more of the blooded or gravid females have been caught with Heleids of the genus *Culicoides* attached to their abdomens. In the daytime this species exhibits very little activity, never attempting to bite the collector, even though the density of the mosquitoes may be very high. When disturbed they will fly out a few inches or feet and again come to rest. The resting position is at an angle of approximately 60 degrees to the surface.

Nocturnal activity during the rainy season usually starts around 6:30 PM and by 7:00 or 7:30 PM all the adults have left their resting places. In the morning they have been observed to return to their roosts between 6:00 and 6:30 AM. Biting records and collections have been made at night in areas with a very high density of this species and a rather low density of *A. farauti*. *A. lungae* formed only 2 per cent of the total anopheline catch, while *farauti* comprised the remaining 98 per cent. In routine night catches near human dwellings conducted for a period of over a year in the Lunga area of Guadalcanal where this species is common, *lungae* accounted for less than 2 per cent of the total anopheline collections. No specimens of this species have to date been taken in native villages. Microscopic examination of the blood from females collected on tree trunks within a half mile of human dwellings revealed that approximately 70 per cent of the blood meals are nonmammalian in origin, the red blood cells being nucleated. There is no conclusive evidence at the present time as to the origin of the mammalian blood found in the stomachs of these mosquitoes. Precipitin tests, made on a small number of stomach contents, were inconclusive. It is felt that probably the majority of such blood is not of human origin as in addition to a variety of birds, a number of species of bats, a marsupial, many pigs, and occasionally cattle are present in the areas where *lungae* is abundant, and, as pointed out above, even with a high density of this species, it is not attracted to humans. It is possible that during the rainy season when the species is flushed into the coastal plain, relatively more available human hosts and scarcer natural hosts may induce *lungae* to feed on man. The experience on Guadalcanal during the last rainy season would seem to indicate that this takes place only locally.

Anopheles (*Myzomyia*) *punctulatus* Doenitz

1901. *Anopheles punctulatus* Doenitz, Insekten Boerse. **18:** 36 (♂ and ♀). **Type locality:** New Guinea; Bismarck Archipelago. **Types:** unknown.

Adult Female.—A medium-sized, speckled anopheline, scales present on scutum, apical half of labium light. Size medium, length of wing 3–4 mm.

Head: Usual ornamentation on front, vertex and occiput. Antennae normal, with minute white scales on torus; patch of light scales on first flagellar segment. Palpi as long as proboscis; second morphological segment with narrow apical light ring, remainder usually black; third segment usually with narrow apical light ring, a broad subapical light band, separated by narrow dark ring; occasionally pale markings fused to form a very broad apical pale area; fourth and fifth segments broadly light with narrow basal black rings. Apical third to half of labium entirely pale except for a narrow subapical dark ring and scattered dark scales ventrally; subapical ring occasionally absent. Labella light. Buccopharyngeal armature of several teeth forming a single row; teeth of similar character, separated by intervals; apices of teeth deeply serrated; base of each tooth with bulla and a stout spine on each side.

Thorax: Scutum light yellow-brown to dark brown, darker eye spots anterior to scutal angle and dark prescutellar space. Anterior promontory with sparse elongate erect white scales in center, somewhat shorter, broader scales in lateral tuft, light above, dark below. Remainder of scutum with vestiture of broad, recumbent, yellowish-white scales and golden hairs; scales

somewhat longer in front and above wing base. Scutellum with disc dark; a few whitish scales, smaller than on scutum. Scutal and scutellar bristles golden brown. Prothoracic lobes dark with broad erect black scales dorsally. Pleural integument dark brown with usual light areas. Spiracular bristles absent; propleurals 2–3, rarely 1 or 4–6; lower sternopleurals usually 4 light hairs; upper sternopleurals 5–7 hairs; prealars a group of 4–6 light hairs; subalars a group of 4–10 light hairs; lower mesepimerals absent. Patch of 6–7 broad recumbent light scales on upper sternopleura, similar patch of 5–6 scales on lower sternopleura; rest of pleura bare. Halteres light at base, dark-scaled on knob.

Wing: As figured for *A. p. punctulatus* by Ross and Roberts (1943) but extremely variable. Costal border with four large dark spots; small dark sectoral spot usually present on costa between basal and median dark spots; small dark spots on vein 1 below median dark spot may coalesce, also small dark spots on vein 1 below preapical dark spot, giving much darker appearance to wing. Veins 2–6 with numerous small dark spots. Light scales yellowish-white. Fringe dark, light spots usually at apices of all veins.

TABLE. 1.—*Summary of diagnostic measurement* data for pupae of* punctulatus *series*

Species	Hair 8 of Cephalothorax Length				Hair 5 of Ab.† II Length			
	Range	Ave.	% under 105 mic.	% 105 mic. or over	Range	Ave.	% under 159 mic.	% 159 mic. or over
punctulatus	95–166	111	2.3	97.7	119–238	181	2.9	97.1
koliensis	51–88	69	100.0	0.0	74–153	124	100.0	0.0
farauti	68–116	82	97.4	2.6	98–164	150	99.4	0.6

Species	Hair 1 of Ab. IV Length				Hair 1 of Ab. VI Length			
	Range	Ave.	% under 27 mic.	% 27 mic. or over	Range	Ave.	% under 104 mic.	% 104 mic. or over
punctulatus	10–34	17	99.5	0.5	102–194	145	1.7	98.3
koliensis	20–71	43	1.6	98.4	51–119	75	99.5	0.5
farauti	17–55	28	33.4	66.6	71–180	109	22.6	77.4

Species	Hair 1 of Ab. VII Length			
	Range	Ave.	% under 111 mic.	% 111 mic. or over
punctulatus	115–204	152	0.6	99.4
koliensis	64–112	85	99.5	0.5
farauti	78–170	118	38.0	62.0

* Measurements in microns.
† Ab. stands for abdominal segment.

Legs: Light brown to black with yellowish-white markings. Front femora swollen in basal half, light at base, speckled and blotched with light along anterior and posterior surfaces; dorsal surface of tibiae with evenly spaced small light spots, ventral surface light, anterior surface dark, posterior surface speckled with light areas the majority of which connect with the light ventral area; first tarsal segment with light apex and variable number of light spots which expand laterally and ventrally, reducing the dark areas on ventral surface; second and third segments light at base and apex, central dark area may be greatly reduced ventrally; fourth segment light at base and usually apex; fifth segment light or dark; segments two to four may have light spots in central dark area; ventral surfaces of all segments usually much lighter than dorsal. Middle and hind femora and tibiae speckled. First segment of middle and hind tarsi similar to corresponding segment of front tarsi; second, third, and fourth segments with narrow apical light bands; fifth usually all dark; second and third segments frequently with one or more light spots in central dark area; ventral and posterior surfaces of segments one and two usually lighter.

Abdomen: Integument dark brown; vestiture of golden hairs, denser on posterior segments. Scales absent on tergites I to V and sternites I–VI, a few scales on tergites VI and VII and sternite VII; rather dense scaling on tergite and sternite VIII. Cerci with dark scales at base, light on apex.

ADULT MALE.—Labium with at least two or three, usually numerous, ventral light spots, frequently with large patches of light scales. Second morphological segment of palpus with light patch dorsally in basal half; articulation between second and third segments without scales,

TABLE 2.—*Summary of diagnostic measurement data for pupae of* lungae *series*

Species	Hair 1 of Ab. III Length				Hair 1 of Ab. IV Length			
	Range	Ave.	% under 17 mic.	% 17 mic. or over	Range	Ave.	% under 17 mic.	% 17 mic. or over
lungae	6–17	12	95.0	5.0	6–17	12	100.0	0.0
solomonis .	10–28	19	12.2	87.8	17–41	24	4.2	95.8

Species	Hair 1 of Ab. V Length			
	Range	Ave.	% under 25 mic.	% 25 mic. or over
lungae	10–24	14	100.0	0.0
solomonis .	27–75	58	0.0	100.0

light-colored; third segment dark on basal fifth, light-scaled to apical fourth, remainder dark except light preapical area extending laterally to apex or apex light; segments four and five narrowly dark at or near base, dark area extending on outer surface usually to apex of segments, remainder light. Claws normal.

Genitalia: As figured by Ross and Roberts (1943).

TABLE 3.—*Branches of diagnostic hairs of pupa*

Species	Ab. I				Ab. II			
	Hair 6		Hair 9		Hair 5		Hair 10	
	Average	Range	Average	Range	Average	Range	Average	Range
hollandi	9	7–11	3	2–4	6	5–9	7	4–11
solomonis	7	5–9	2	1–4	3	2–5	6	5–8
lungae	6	5–7	1	1–2	3	1–4	7	5–10
punctulatus	5	3–8	1	1–1	1	1–2	18	7–38
koliensis	5	4–6	1	1–1	1	1–1	5	2–9
farauti	4	1–5	1	1–2	1	1–3	7	4–16

Species	Ab. III				Ab. IV			
	Hair 5		Hair 10		Hair 2		Hair 10	
	Average	Range	Average	Range	Average	Range	Average	Range
hollandi	8	5–10	10	7–14	3	2–4	8	6–10
solomonis	3	2–5	4	3–5	2	1–2	4	2–5
lungae	2	1–2	4	3–6	2	1–3	3	2–5
punctulatus	1	1–3	10	4–16	1	1–2	6	4–8
koliensis	1	1–2	10	7–13	1	1–2	7	5–9
farauti	1	1–2	8	5–11	1	1–1	6	3–8

Species	Ab. V				Ab. VI			
	Hair 10		Hair 2		Hair 6		Hair 10	
	Average	Range	Average	Range	Average	Range	Average	Range
hollandi	5	4–7	3	1–4	7	5–11	5	3–7
solomonis	3	2–4	2	2–3	3	2–4	2	2–4
lungae	3	2–4	2	1–3	4	3–5	2	1–4
punctulatus	3	2–4	2	1–3	4	3–5	1	1–3
koliensis	4	3–6	1	1–1	4	2–5	2	1–3
farauti	3	1–6	1	1–2	4	3–6	2	1–4

Species	Ab. VII				Ab. VIII			
	Hair 6		Hair 8		Hair 10		Hair 8	
	Average	Range	Average	Range	Average	Range	Average	Range
hollandi	7	4–8	6	3–10	5	4–6	4	3–7
solomonis	3	1–4	5	2–8	2	1–4	2	2–3
lungae	4	2–6	4	3–6	1	1–2	2	2–3
punctulatus	4	3–5	4	2–5	1	1–2	2	1–3
koliensis	4	3–5	3	2–4	1	1–2	3	2–4
farauti	4	3–6	3	2–4	1	1–2	2	1–3

PUPA.—Tables 1 and 3 contain the data for the more important hair counts and measurements of this species.

Cephalothorax: Trumpet pale, with only slight pigmentation in the central area between the apex and the basal notch, offering little contrast to the cephalothorax. Index of width to length of head shield 1 : 0.90 (range 0.81–1.0). External margin of palpal case usually angulate. Internal margin of median keel sharply outlined. Metanotum with a distinct, smooth median bar, bounded laterally by a crease which is incomplete posteriorly. Details of chaetotaxy not noticeably different from those of *solomonis*.

Abdomen: On segment II, hair 2 on or very near to a longitudinal line through hair 3; on segment IV, hair 5 external to hair 8; on segments VI and VII, hair 2 distinctly anterior to the posterior margin of the segment. Lateral spine (hair 1) of segment IV approximately equal to the length of that of segment III; lateral spine of V several times the length of that on III, and two-thirds to three-fourths the length of that on VI; lateral spines of V–VII (Fig. 26, H) rarely branched, frayed or with irregular outline, extremely elongate and slender. Similar to *solomonis* in the remaining details of structure and chaetotaxy.

Paddle: Midrib with external margin distinct, completely without granulations in over 50 per cent of the specimens examined, when granulations present seldom completely filling the area of the midrib but arranged in slender skeins along its length. Buttress well developed. Fringe of fine pale hairs extending from center of external margin to a short distance internal to hair 12, longest just before this hair. Paddle hair (hair 12) straight or curved, rather stout, darkly pigmented.

Genitalia: Apex of lobes in male shaped as shown in Fig. 17. Female genitalia as in Fig. 21.

LARVA.—Anterior clypeal hairs unbranched, the outer half or less the length of the inner. Posterior clypeals single, usually long enough to reach the tubercles of the anterior clypeals. Occipitals long, inner single or double; outer 1–3(rarely 4)-branched. Tubercles of prothoracic hairs 1 and 2 small, unpigmented, separated from one another; stem of hair 1 slender, at most slightly enlarged basally. Long thoracic pleural hairs all single. Hair 1 of abdominal segment I with short, hair-like branches. Palmate hairs present on segments II–VII, all small, especially on segment II; filament of leaflet almost as long as the shaft. Hairs 2 and 6 of abdominal segments IV and V usually 3-branched.

Taxonomic Discussion.—A. punctulatus, farauti, koliensis, and *moluccensis* form a closely related complex of species in the group *Neomyzomyia*. The combination of the presence of scales on the disc of the scutum, the absence of scales from all but the terminal abdominal segments, the speckling of the legs, and the broad apical light bands on the last two palpal segments (3rd palpal segment variable) separate the *punctulatus* series from all other *Neomyzomyia* in the Oriental and Australasian regions. The larvae can usually be recognized from other *Neomyzomyia* with unbranched pleural hairs by the single or frayed widely spaced inner clypeals, by the single, frayed, or occasionally branched outer clypeals, which are never minute, and by the posterior clypeals which at most barely project beyond the margin of the head. *A. watsoni* and *hackeri* exhibit similar characters but can be separated by the position of the clypeal hairs, the bases of the inner, outer, and posterior clypeals on one side forming an equilateral, scalene or isosceles triangle.

The larva of *moluccensis* from New Guinea appears to be similar in structure to that of *farauti,* in that the tubercles of the prothoracic submedian hairs are fused and hair 1 of abdominal segment I is distinctly palmate with flattened leaflets. However, according to Swellengrebel and Rodenwaldt (1932) the adults of this species have the small light ventral patch of the labium which is characteristic of *koliensis*. *A. koliensis* and *moluccensis* larvae are easily separated from one another. Until comparison can be made with the type of *moluccensis* and with reared material from New Guinea, the exact relationship of *moluccensis* to *farauti* and *koliensis* cannot be determined.

It has been suggested that hybridization occurs between various members of the *punctulatus* series. Although no conclusive cross-breeding experiments have been made, the fact that the progeny of all species except of one female *koliensis* bred

true indicates that hybridization in the area in question does not normally occur in nature even though the species exist side by side. *A punctulatus, farauti,* and *koliensis* can be separated from one another in the adult, pupal and larval stages. Therefore, we prefer to treat these forms as species, and not as subspecies of *punctulatus*.

Distribution.—GUADALCANAL. North coast: Tenamba, Umasami, Tassafaronga, Bonegi, Poha, Kokumbona, Matanikau, Lunga, Tenaru, Koli, Tetere; Southwest coast: West Cape. BOUGAINVILLE. Empress Augusta Bay (A. B. Gurney).

Biology.—On Guadalcanal, this species is restricted to the valleys of the larger streams and rivers, usually occurring in the vicinity of native villages away from the coast. Larvae have been collected most frequently exposed to sunlight in road ruts and other temporary pools such as depressions and footprints in native trails. The margins of streams and sloughs in exposed situations and pot holes in drying stream beds are also utilized occasionally, particularly during the dry season. The pools in which this species occurs may be entirely free of vegetation and flotage and frequently are very muddy, or may have marginal herbaceous vegetation and dense algal growth. *A. punctulatus* has a very decided preference for breeding in sunlight, but is also found in partial shade.

During periods of dry weather this species almost disappears as its chief breeding places are greatly reduced. At such times it resorts to breeding in streams. After an occasional heavy rain it appears in large numbers in temporary pools. During the rainy season *punctulatus* extends its range into the coastal plain near the mouths of the rivers and utilizes the same breeding places as *farauti*.

The larvae and pupae can usually be recognized in the field by the uniformly light opaque coloration of the body. They are extremely active, especially when crowded in small breeding places without vegetation and seem to spend a great deal of time under water in search of food.

Some information on the length of the aquatic cycle of *punctulatus* and *farauti* under natural conditions was obtained on Guadalcanal in early March 1944. With relatively low water temperature (70°–91° F.), high rainfall and unfavorable food supply (old oil film and euglenoid film), the larval stage was observed to last five days. Approximately one day was required for the completion of each of the first three instars, and two days for the fourth instar. As the eggs took slightly less than two days to hatch and the pupal stage lasted approximately 20 hours, the entire aquatic cycle from oviposition to emergence of the adults was accomplished in less than eight days. Under more favorable conditions it undoubtedly is reduced to less than a week. The possibility that eggs are laid in mud was indicated by the appearance of first instar larvae in isolated temporary pools less than a day after they had become filled with water. Puddles with larvae and pupae were observed to dry completely; when these were filled two days later fourth instar larvae and pupae appeared. It would appear from these observations that *punctulatus* and *farauti* are well adapted to breeding in temporary pools and may be able to survive at least short periods of dry weather in the egg and possibly also the larval and pupal stages. Further indications of the short aquatic cycle of *punctulatus* and its adaptation to breeding in temporary pools were obtained from observations on a colony of this species maintained for a period of three months during the dry season. Gravid females would not lay eggs in small water con-

tainers, but scattered them on the floor of the cages. Eggs were collected in large numbers on moist filter paper placed on the bottom of the cage. Hatching took place in less than two days after oviposition, all the first instar larvae appearing in less than one hour of each other. A strikingly uniform rate of development of the larvae was observed in this species and was different from all other local anophelines. At any one time in a batch of the same age, the larvae would be of the same instar and size, taking from six to seven days to pupation with adequate food supply.

The short time required for *punctulatus* and *farauti* to complete the aquatic cycle in nature is of considerable interest in relation to control operations directed against these species.

Although females of *punctulatus* feed readily in captivity, they do not appear to attack man often in nature on Guadalcanal. Less than one per cent of the total anophelines collected in routine night catches for a period of over a year have been *punctulatus*. Special efforts were made to collect adults in native villages and in the open in areas of high larval densities of this species on the northwest coast of Guadalcanal with the result that a single blooded female was obtained in a native hut, while *farauti* was collected at will in the open as well as in dwellings. On the northeast coast *punctulatus* has been collected in native camps and villages, but in smaller numbers than *koliensis* or *farauti*.

The diurnal resting places of the adults of this species are not known on Guadalcanal. They have been searched for on stream banks, in jungle areas near breeding places, and in the vicinity of native villages.

Anopheles (*Myzomyia*) *farauti* Laveran

1902. *Anopheles Farauti* Laveran, C. R. Soc. Biol. Paris **54**: 908 (♀ ♀ only). **Type locality:** Faureville, Ile Vate, New Hebrides. **Types:** unknown.
1927. *Anopheles* (*Myzomyia*) *punctulatus* Doenitz. Buxton and Hopkins, Res. in Polynesia and Melanesia, p. 67.
1929. *Anopheles punctulatus* Doenitz. Paine and Edwards, Bull. Ent. Res. **20**: 304.
1931. *Anopheles punctulatus* Doenitz. Senevet, C. R. 2e Congr. Intern. Paludisme, Instit. Pasteur, Alger. **1**: 109.
1944. *Anopheles punctulatus farauti* Laveran. Knight and Farner, Proc. Ent. Soc. Wash. **46**: 132.
1944. *Anopheles farauti farauti* Laveran. Knight, Bohart and Bohart. Keys to the Mosquitoes of the Australasian Region. Nat. Res. Council, Wash., p. 11.

Adult Female.—In general as in *punctulatus*. Labium entirely dark-scaled except for narrow light ring at extreme apex; a few light scales rarely present on basal half. Third morphological palpal segment usually with narrow apical light ring separated from larger subapical light area by dark ring; these pale markings, especially the subapical spot, frequently reduced, sometimes subapical completely absent. Small dark sectoral spot usually present on costa between basal and median dark spots. Buccopharyngeal armature as in *punctulatus*.

Adult Male.—In general as in *punctulatus*. Labium entirely dark-scaled except for narrow light ring at extreme apex. Small dark sectoral spot usually present on costa between basal and median dark spots.

Genitalia: Similar to those of *punctulatus*.

Pupa.—The three terminal abdominal segments of this species were figured by Buxton and Hopkins (1927) from New Hebridean specimens. Senevet (1931) described and illustrated the dorsal and ventral chaetotaxy of an anopheline from Iles Salomon (Solomon Islands ?) which he identified as *punctulatus*. However, from the incomplete figure given by Senevet, it appears that his specimens were *farauti*. Tables 1 and 3 contain the data for the more important hair counts and measurements made for this species. Similar to the pupa of *punctulatus* except for the following:

Cephalothorax: Trumpet orange-brown, contrasting noticeably to cephalothorax, pigmented area extending basad from the apex at least to the basal notch. Index of width to length of head shield 1 : 0.88 (range 0.65–1.11).

Abdomen: On segment IV, hair 5 internal to or in line with hair 8. Lateral spine (hair 1) of segment IV approximately twice the length of that of III; lateral spines of segments V–VI may occasionally have one or more minute lateral branches, or even be terminally frayed; lateral spine of segment VII (Figs. 26, D and E) elongate, stout, acutely tapered, usually branched or frayed; frequently those without obvious branching give the impression of possessing branches that are not separated from the shaft.

Paddle: Midrib usually evenly granulated.

LARVA.—Anterior clypeal hairs usually frayed; outer at least half the length of the inner; posterior clypeals long, extending beyond the anterior tubercles, usually single, sometimes 2-branched. Occipital hairs short; inner 1–2(rarely 3)-branched, outer 1–4(rarely 5–6)-branched. Tubercles of prothoracic hairs 1 and 2 long, pigmented, and almost always fused; stem of hair 1 swollen basally. Hair I of abdominal segment I a definite palmate tuft, with flattened, notched leaflets. Palmate hairs also present on segments II–VII; those on segment II pigmented and only slightly smaller than those on segment III. Leaflets with long filaments, sometimes almost the length of the shaft. Hairs 2 and 6 of abdominal segments IV and V usually double.

Variation.—On Guadalcanal in areas where there is little chance for human contact such as near abandoned native villages or campsites and beyond the edge of the controlled territory, the larvae of *farauti* are somewhat different from those reared in the laboratory in that the outer clypeals and outer occipitals exhibit heavier branching. A similar condition exists in more darkly pigmented larvae collected in the control area. Adults bred from larvae collected in such areas do not feed readily on humans. This seems to indicate the presence on Guadalcanal of a "wild" strain of *farauti.*

Taxonomic Discussion.—See under *A. punctulatus.*

Distribution.—NEW HEBRIDES: EFATE. Faureville (Laveran, 1902), Teuma and Havannah Harbor (Buxton and Hopkins, 1927), generally distributed along the coastal areas. ESPIRITU SANTO. Big Bay (Buxton and Hopkins, 1927), generally distributed along the southeastern and southern coastal area. AORE. MALO. TANNA. Whitesands (Buxton and Hopkins, 1927). MAI. (Buxton and Hopkins, 1927). MALEKULA. Tisman (Buxton and Hopkins, 1927), Port Sandwich. SOLOMON ISLANDS: GUADALCANAL. Generally distributed along the entire coast, has been collected on the north coast from Marovovo to Aola Bay, and at West Cape on the southwest coast. MALAITA (S. M. Lambert, J. R. Douglas). TULAGI. FLORIDA. SAVO. RUSSELL ISLANDS (W. G. Downs). NEW GEORGIA GROUP (W. G. Downs, J. G. Franclemont). BOUGAINVILLE. Empress Augusta Bay (A. B. Gurney). TREASURY ISLANDS (J. H. Paullus).

Biology.—The natural breeding places of this species consist primarily of river and stream margins with vegetation, springs, wells, seepage areas, taro gardens, ponds, lagoons, and swamps, all in open coastal areas. Even when these breeding areas are greatly reduced *farauti* does not invade such habitats as undisturbed dense canebrake marshes, or large open sunlit swamps in the jungle filled with floating and emergent vegetation. Normally, the breeding does not extend far from settled areas, usually not more than a mile, unless the jungle is cleared. However, breeding will often persist in areas once occupied, but later abandoned. Following heavy rains any of the very numerous temporary pools formed in the open coastal areas are utilized by this species. These may be natural or man-made water-holding depressions such as puddles, hog wallows, ruts, holes, hoofprints, bases of uprooted trees, borrow pits, poorly graded ditches and occasionally holes in coral above high tide mark. Frequent floods of the larger streams and rivers eliminate them as important breeding places during periods of high rainfall. When the species is denied breeding areas on the ground or when it becomes extremely abundant, it

resorts to breeding in artificial containers to a limited degree. At such times it has been collected in boats, tanks, oil drums, water collections in canvas, and even small tin cans.

The aquatic stages of *farauti* have a definite preference for sunlight, but are very frequently collected in large numbers under partial shade, rarely being found in very dense shade. This species tolerates a wide range of conditions, utilizing all types of water from highly turbid to clear spring water, from stagnant, foul or brackish to fresh rain water. It is not found in water collections in plants, such as leaf or frond axils, cocoanut shells, cacao pods, or tree holes, nor does it occur in water with high organic content, such as is found in jungle swamps. In almost all cases, breeding in extensive water areas is associated with flotage and emergent or surface vegetation. However, in small confined places such as pools, puddles, and road ruts, larvae will commonly be found on the open surface.

A. farauti is frequently associated with *punctulatus* where the latter is present in temporary pools during the rainy season. In the dry season it is found together with *B. hollandi* and *A. lungae* along the more exposed stream margins. The culicines most frequently associated with *farauti* are *Culex annulirostris* Skuse, *C. basicinctus* Edw. (New Hebrides), *C. squamosus* (Taylor), (Solomons), *C. jepsoni* Theo., *C. pullus* Theo. (Solomons), and during the rainy season various species of flood-water *Aedes*.

Larvae collected in the field frequently have a dark pigmentation mottled with light areas, but they may be just as light as those of *punctulatus* or greenish depending on the type of breeding place. Usually the antennae are darkly pigmented.

The aquatic cycle appears to be very short. It has been discussed under *punctulatus*.

Adult females enter any type of shelter in search of human blood, and readily bite out of doors. Feeding takes place generally from dusk to dawn. However, a number of daytime biting records have been obtained. In most cases, these attacks were in covered or shaded areas, but a few were made directly in bright sunlight. The flight is relatively quiet and the females are shy and wary in their approach, easily frightened away by movements, but persistent in returning to attack. In houses or tents where lights were present, they will feed most readily in dark corners and on shaded portions of the body. Their bite is noticeably painless to many individuals. Throughout the area covered by this report, except for isolated areas in the Koli district on Guadalcanal, *farauti* is the principal anopheline attacking man. On the northwest coast of Guadalcanal, 98 per cent of anophelines in routine night catches conducted for a period of over a year were this species.

The diurnal resting places for males and females apparently consist of nearly any cool, moist and shaded spot. Adults of *farauti* have been collected on Guadalcanal resting on buttressed tree trunks and under logs in swampy jungle areas in association with *lungae* and *solomonis,* but never in large numbers. In the New Hebrides they have been found among roots of banyan trees, weeds and grass, in open barrels, tin cans and wooden boxes, on shaded, moist earthen walls of pits and holes, and in moist situations beneath large felled logs. Resting blooded females may be collected at will in occupied houses and native huts, the sites being generally dark and somewhat secluded, such as in the folds of clothing and mosquito nets, on the undersurface of furniture, rafters and hanging objects and upon walls and low ceilings. They are commonly found from the ground surface to a height

of seven feet. On Guadalcanal they have been collected in native huts and tents under extremely high temperature in the middle of the day.

No definite information is available on flight range. Our records show several instances of adults having been captured at least one mile from the nearest known breeding area. On Espiritu Santo a blooded female was found by R. H. Daggy on a boat 600 yards off shore in the channel. Adults are commonly taken up to 400 yards from breeding areas.

Although no comprehensive data are available on the longevity of *farauti,* several females were kept alive in a small laboratory cage for approximately 35 days. The males, which were introduced at the same time, survived less than two weeks. Both insectary and cage mating have been obtained with this species. Although on Espiritu Santo and Efate colonies were readily established, such attempts on Guadalcanal met with great difficulty. This may be due in part to that fact that larvae were collected along the margin of the control area in places where human blood was not available. Under the section on variation above, the differences in the morphology of larvae found in such areas are discussed and the possibility of the existence of a "wild" strain of *farauti* is suggested.

Anopheles (Myzomyia) koliensis Owen

1945. *Anopheles punctulatus koliensis* Owen, 1945. **Type locality:** Koli area, Guadalcanal, **Types:** U. S. National Museum.

ADULT FEMALE.—In general as in *punctulatus.* Apical third of labium with ventral area of pale scales; usually a well-defined ventral patch, which may extend dorsally in form of pale band at its anterior extremity; rarely pale markings reduced to a few scales ventrally; very rarely apical third dark-scaled as in *farauti* or extensively pale-scaled as in *punctulatus;* dorsal pale area when present not as extensive as in *punctulatus;* a few pale scales in basal half of labium may be present. Usually no small dark sectoral spot on costa between basal and median dark spots. Dark coloration of legs usually more extensive than in *punctulatus,* extending to ventral surface of tarsal segments. Buccopharyngeal armature as in *punctulatus.*

ADULT MALE.—In general as in *punctulatus.* Labium usually entirely dark-scaled except for a narrow apical light ring at extreme apex. Wings and legs as in female *koliensis.*

Genitalia: similar to *punctulatus.*

PUPA.—The pupa of this species is quite similar to that of *farauti.* Tables 1 and 3 give the data for the hair counts and measurements that have diagnostic value.

Cephalothorax: Index of width to length of head shield 1 : 0.88 (range 0.76–1.0).

Abdomen: Hair 5 of segment IV is frequently (35 per cent) external to hair 8, whereas in *farauti* it is in line or internal to hair 8 (91 per cent). Lateral spine of segment 7 (Fig. 26, F) stout, acutely tapered from base, only rarely branched, but when so of a distinctive type (Fig. 26, G).

Paddle: Midrib incompletely granulated, or occasionally granules entirely missing.

Genitalia: Apices of lobes in male distinctively shaped (Fig. 18).

LARVA.—Anterior clypeal hairs either simple or slightly frayed; outer half or more the length of the inner; posterior clypeals single and very short, not extending to the tubercles of the anterior hairs. Occipital hairs short; inner 2–4-branched; outer usually 5–9-, occasionally 3–4-branched. Tubercles of prothoracic hairs 1 and 2 usually separated; stem of hair 1 swollen. All long hairs of thoracic pleural groups single. Hair 1 of abdominal segment I with long, narrow, hair-like branches. Palmate hairs present on segments II–VII, those on segment II only slightly smaller than those on segment III. Filament of leaflets almost as long as the shaft. Hairs 2 and 6 of abdominal segments IV and V usually 2, sometimes 3-branched.

Taxonomic Discussion.—See under *A. punctulatus.*

Distribution.—GUADALCANAL. North coast from Malimbu River east to Aola Bay (W. B. Owen); absent from the northwest coast.

Biology.—Owen collected the larvae of this species in water exposed to sunlight from temporary pools in grasslands and along the edge of the jungle in association with *farauti* and once in association with both *farauti* and *punctulatus.* They were scarce in comparison with the other anophelines collected. The adults are

strongly anthropophilic. Where blooded females have been collected in the daytime resting in native huts and tents they have formed 90 per cent of the anopheline population. During the dry season they become active about 9:00 PM, the greatest activity occurring after midnight and continuing until daylight (W. B. Owen).

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Explanation of Plates

Plate I—Egg and Adult

Fig. 1. *A. solomonis*—Wing.
Fig. 2. *A. solomonis*—Head, proboscis and palpus.
Fig. 3. *B. hollandi*—Male genitalia.
Fig. 4. *B. hollandi*—Lateral aspect of claspette.
Fig. 5. *A. lungae*—Buccopharyngeal armature.
Fig. 6. *A. solomonis*—Buccopharyngeal armature.
Fig. 7. *B. hollandi*—Egg.

Plate II—Larva

Fig. 8. *B. hollandi*—Head of larva.
Fig. 9. *B. hollandi*—Prothoracic submedian group.
Fig. 10. *B. hollandi*—Ventral aspect of left prothoracic pleural group.
Fig. 11. *B. hollandi*—Ventral aspect of left mesothoracic pleural group.
Fig. 12. *B. hollandi*—Ventral aspect of left metathoracic pleural group.
Fig. 13. *A. solomonis*—Ventral aspect of left prothoracic pleural group.
Fig. 14. *A. solomonis*—Ventral aspect of left metathoracic pleural group.
Fig. 15. *A. lungae*—Ventral aspect of left prothoracic pleural group.

Plate III—Pupa

Fig. 16. *B. hollandi*—Dorsal and ventral aspect of metanotum and abdomen.
Fig. 17. *A. solomonis*—Male genitalia.
Fig. 18. *A. koliensis*—Male genitalia.
Fig. 19. *B. hollandi*—Paddle.
Fig. 20. *A. solomonis*—Paddle.
Fig. 21. *A. farauti*—Female genitalia.
Fig. 22. *B. hollandi*—Male genitalia.
Fig. 23. *B. hollandi*—Trumpet.
Fig. 24. *B. hollandi*—Female genitalia.
Fig. 25. *B. hollandi*—Lateral spine (hair 1) of abdominal segment VII.
Fig. 26, A. *A. solomonis*—Lateral spine of abdominal segment VII.
Fig. 26, B and C. *A. lungae*—Lateral spine of abdominal segment VII.
Fig. 26, D and E. *A. farauti*—Lateral spine of abdominal segment VII.
Fig. 26, F and G. *A. koliensis*—Lateral spine of abdominal segment VII.
Fig. 26, H. *A. punctulatus*—Lateral spine of abdominal segment VII.
Fig. 27. *B. hollandi*—Anterior portion of cephalothorax.

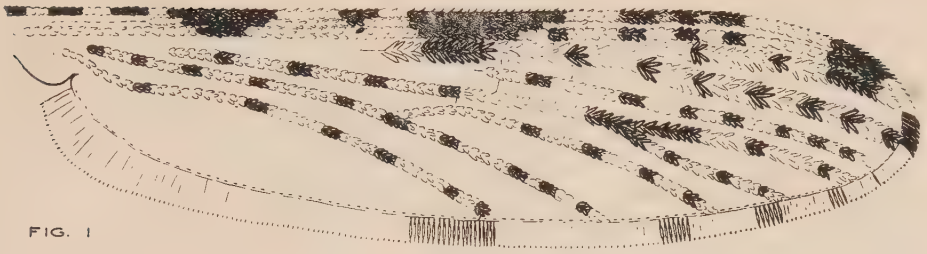

FIG. 1

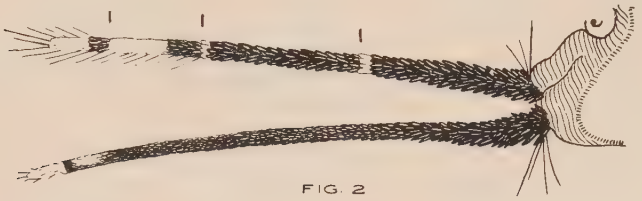

FIG. 2

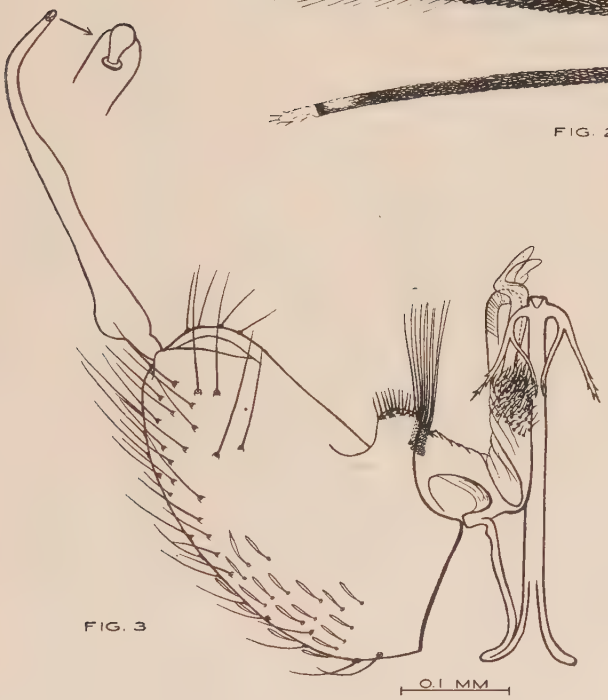

FIG. 3

FIG. 4

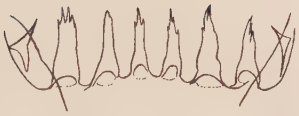

FIG. 5

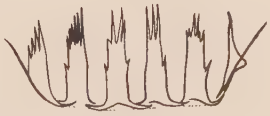

FIG. 6

FIG. 7

PLATE I

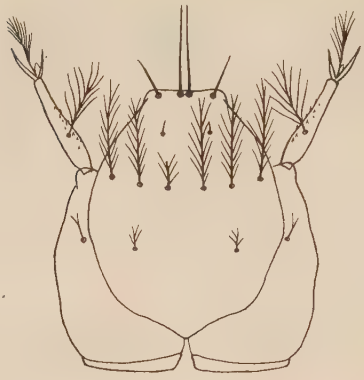

FIG. 8

FIG. 9

FIG. 13

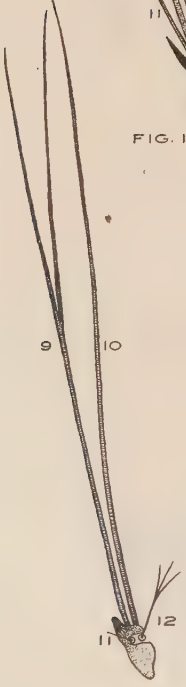

FIG. 14

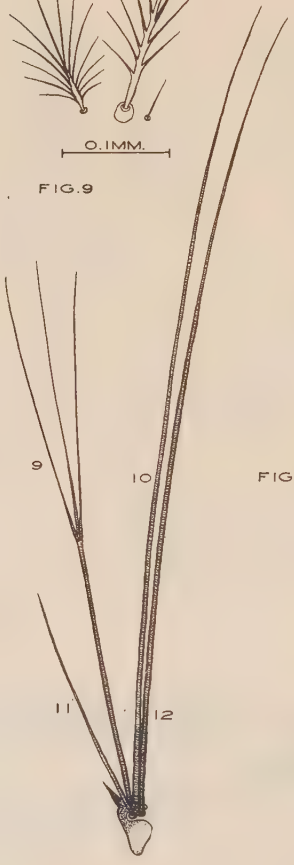

FIG. 15

FIG. 10

FIG. 11

FIG. 12

PLATE II

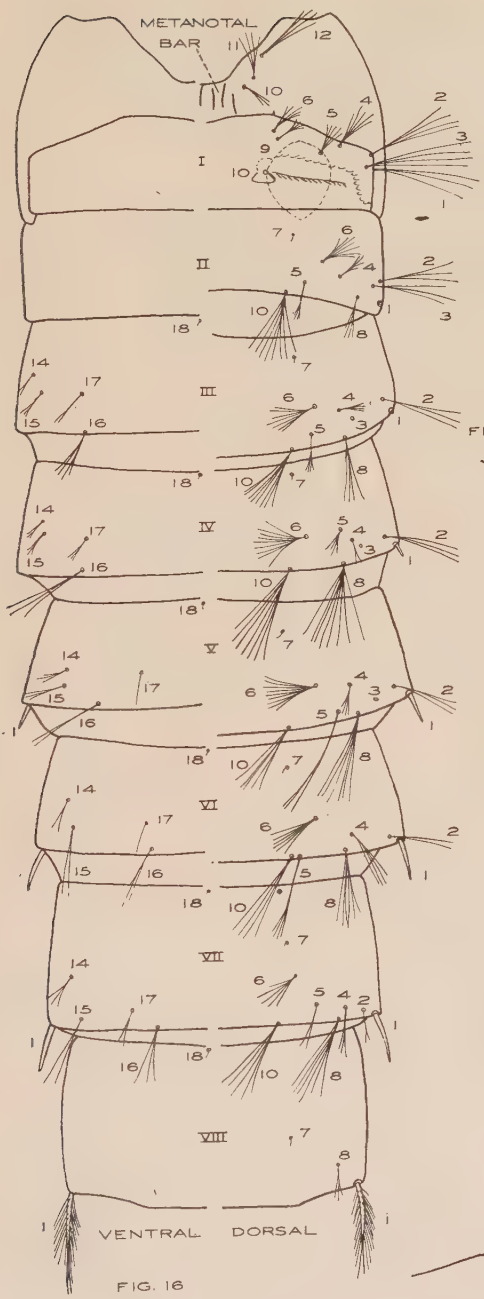

FIG. 16

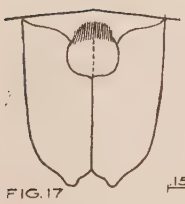
FIG. 17

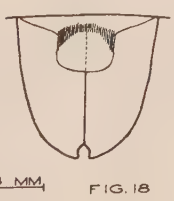

FIG. 18

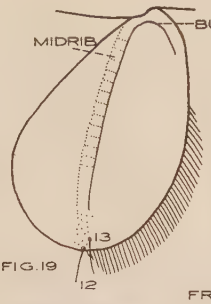

FIG. 19

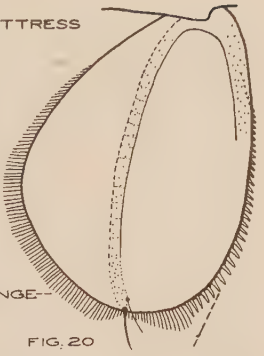

FIG. 20

FIG 21

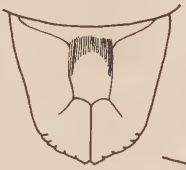
FIG 22

FIG. 23

FIG. 24

FIG. 25

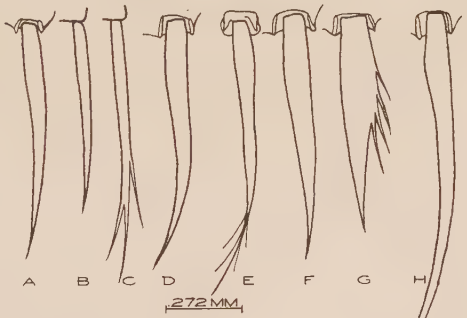

FIG. 26

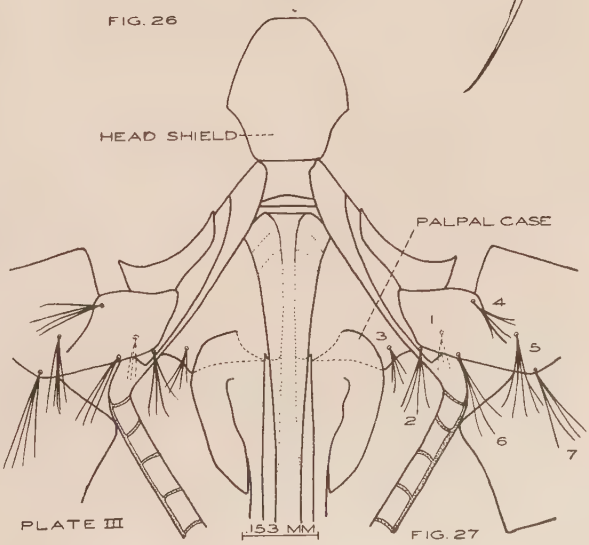

PLATE III
FIG. 27

EXPERIMENTS TO DETERMINE WHETHER INFECTIVE LARVAE OF *WUCHERERIA BANCROFTI* CAN MIGRATE FROM THE ABDOMEN OF THE MOSQUITO INTERMEDIATE HOST

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In the course of dissecting mosquitoes experimentally infected with *Wuchereria bancrofti,* the authors found many specimens containing infective larvae in sites other than the head and proboscis, particularly in the abdominal cavity. It was of practical interest to find out whether or not infective larvae occurring in the abdominal cavity are capable of migrating forward to the proboscis. If free migration within the mosquito could be demonstrated, it could be assumed that infective larvae occurring anywhere within the mosquito could reach the proboscis and thereby be involved in transmission.

In the event that infective larvae occurring within the abdominal cavity do not migrate forward, it should be possible to demonstrate one or more of the following phenomena: That there is some anatomical barrier to the forward progress of the larvae once they have reached the abdomen, that there is some physiological reaction on the part of the mosquito which engulfs or kills the larvae, or that migration on the part of the larvae is indifferent; i.e., there is a general migration away from the thorax, but once the larvae have escaped this area there is no impulse to migrate elsewhere.

No anatomical barrier has been observed. To the contrary, the open-type circulatory system of the mosquito would seem to provide an excellent passage for forward migration of the larvae from the abdomen. There has been no evidence of encapsulation or death of the larvae within the abdomen of mosquitoes that permit development to the infective stage. Finally, mosquitoes dissected shortly after the larvae have become infective contained most of the larvae in the abdominal cavity; whereas, specimens kept several days longer contained fewer larvae in the abdomen with a greater proportion of them in the thorax, head, and proboscis. This would indicate that larval migration is not aimless and that there is a tendency for larvae in the abdominal cavity to migrate forward toward the head.

In view of the above, it was the opinion of the authors that infective larvae of *Wuchereria bancrofti* were both capable of and inclined to migrate out of the abdominal cavity toward the head and proboscis.

There has been little, if any, experimental work to prove or disprove the theories regarding the migration of infective *Wuchereria bancrofti* larvae in the mosquito host. Yamada (1927) suggested that the infective larvae migrated from the thorax to the abdomen and then to the head. Highby (1943), while working with *Dipetalonema arbuta,* actually observed the infective larvae migrating freely from one part of the body to another and from the proboscis back to the abdomen.

To provide data on this point, an experiment was set up to determine whether the larvae are able to make their way out of the abdomen. The technique consisted of transferring infective larvae to the abdomen of a non-infected mosquito. If the

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larvae are capable of migrating and are prone to migrate, it should be possible to recover them in other parts of the body on subsequent dissection of the mosquito.

The operation was a delicate one and resulted in the injury or death of some of the transposed larvae. Small numbers of larvae were used in each test in order to minimize the chances of injuring the specimens by overcrowding in the pipette. After the removal of legs and wings, a short, longitudinal incision was made along the lateral abdominal wall of the recipient mosquito. By means of a capillary pipette, as much of the fluid as possible was removed from the cavity. A drop of saline containing 1 to 7 infective larvae was placed over the wound. As the drop evaporated and grew smaller, the larvae eventually worked their way down into the

TABLE. 1.—*Results of dissection of mosquitoes into which infective larvae of* Wuchereria bancrofti *were implanted*

Experiment number	Recipient species	Number larvae implanted	Time elapsed before dissection (minutes)	Location of larvae recovered			
				Proboscis	Head	Thorax	Abdomen
1	*Psorophora confinnis*	4	20	1*	0	1	2**
2	*Culex tarsalis*	3	30	0	1	1	1
3	*Aedes aegypti*	2	50	0	0	1	1***
4	*Culex quinquefasciatus*	2	35	0	1	0	1
5	"	4	20	0	0	0	3 1**
6	"	4	95	0	0	0	1 3***
7	"	4	40	0	3	0	1
8	*Anopheles quadrimaculatus*	3	55	0	0	1	1*** 1
9	"	2	60	0	0	2	0
10	"	3	60	1	0	1	1**
11	"	2	60	0	0	1	1**
12	"	3	60	0	0	2	1
13	"	3	90	0	0	2	1
14	"	3	60	0	0	3	0
15	"	2	60	0	0	1	1
16	"	2	90	0	0	0	2
17	"	4	60	2	0	1	1***
18	"	4	55	2*	0	2	0
19	"	2	90	0	0	0	2
20	"	2	75	0	0	2	0
21	"	4	70	0	0	1	3
22	"	7	60	0	0	5	1** 1
23	"	3	105	0	0	1	2
24	"	1	100	0	0	0	1
25	"	2	80	0	1	1	0
26	"	3	40	0	0	2	1
27	"	2	30	0	0	1	1

* —One of these larvae was seen in the proboscis within 5 minutes.
** —These were found to be injured.
***—These were found to be dead.

abdomen. When the larvae were slow in entering the abdomen, water had to be added to the drop to prevent the salt solution from becoming too concentrated. Within a few minutes the incision closed over, sealing the larvae inside where they usually could be seen moving about. After a time lapse, usually an hour, the mosquito was separated into abdomen, thorax, head, and proboscis. These parts were then examined for the presence of larvae.

Of a total of 27 mosquitoes in each of which at least one live larva capable of migration was recovered upon dissection, 22 contained larvae that had migrated from the abdomen (See Table 1). Of the total of 68 active larvae recovered from all the mosquitoes dissected, 32 had migrated as far as the thorax and an additional 12 as far as the head or proboscis. Twenty-four active larvae were found in the abdomen and apparently had not migrated from that site.

It would appear that the amount of time required for the migration of the larvae to any particular part of the body was irregular. In one instance by means of a strong light a larva was seen in the proboscis 5 minutes after its introduction into the abdominal cavity. In another specimen larvae were found only as far as the thorax after 105 minutes.

SUMMARY AND CONCLUSIONS

Inoculations of infective larvae of *Wuchereria bancrofti* were made into the abdomen of previously uninfected mosquitoes to determine whether such larvae could migrate from this site to other parts of the body, particularly to the proboscis. The dissection of 27 mosquitoes so inoculated indicated that in 22 specimens the larvae had migrated from the site of injection. Of the total of 68 live larvae recovered, 32 had migrated as far as the thorax and an additional 12 as far as the head or proboscis.

The results indicate that the infective larvae of this species are capable of moving freely from the abdomen to other parts of the body of the mosquito intermediate host and that there is a definite tendency for such migrations to take place. Therefore infective larvae occurring anywhere in the body of the mosquito are potentially capable of transmitting infection to man.

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THE MIRACIDIUM OF *PROTEROMETRA MACROSTOMA* (FAUST) HORSFALL 1933[1]

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In connection with another study on *Proterometra macrostoma* (Faust) Horsfall 1933, eggs with active miracidia were obtained. In trying to determine the number of flame cells of the unhatched miracidium, some were observed to hatch on the slide. By alternating treatment with salt and tap water and thus changing the osmotic pressure and slight coverglass pressure, several miracidia were obtained, which moved about actively.

DESCRIPTION

The miracidium of *Proterometra macrostoma* is relatively large and pear-shaped, wider at the anterior end (Fig. 1). It is non-ciliated and has prominent bristle plates. At the anterior end these are arranged in five large patches of large bristles, (Figs. 2, 4) each with an associated band of smaller bristles (Figs. 2, 5) arranged in a somewhat spiral fashion. At the posterior end and extending nearly to the anterior patches are four longer bands of smaller bristles (Fig. 2), also arranged in a spiral fashion. A preparation impregnated with silver nitrate to show the boundaries of the dermal plates confirmed this observation, as there were five well-defined anterior plates and four posterior ones (Fig. 3), all rather widely separated from each other. Unfortunately only one miracidium was obtained in this preparation. The break and spur on one of the posterior plates may be (and probably is) an abnormality, but it was impossible to check. In the unhatched miracidium both Dickerman (1934) and Horsfall (1933, 1934) described only four anterior bristle patches. In the type of preparation studied, one bristle patch might have been overlooked.

The anterior central portion of the miracidium contains the so-called "primitive gut" showing four nuclei arranged in a linear series; laterally and posteriorly the miracidium is filled with germinal tissue; a single pair of flame cells is present (Fig. 1).

ACTIVITY

Just before hatching, the miracidium becomes very active within the egg shell, moving around and rubbing against the inside of the shell. This activity is especially noticeable at the anterior end, with an alternate extension and withdrawal of the anterior proboscis-like structure and the anterior bristle patches. When the proboscis-like structure is withdrawn to its full extent (a, Fig. 1) the anterior large bristles point forward. The miracidium seems to swell and fill the shell more completely, pushes or stretches as if to force off the operculum, then finally pushes it off and creeps or flows out. When entirely free from the shell the miracidium moves about by a creeping movement very similar to that of an earthworm, elongating and contracting, the anterior end with its bristles acting as an exploring organ and possibly, in its contact with the substratum, aiding in locomotion. Since the

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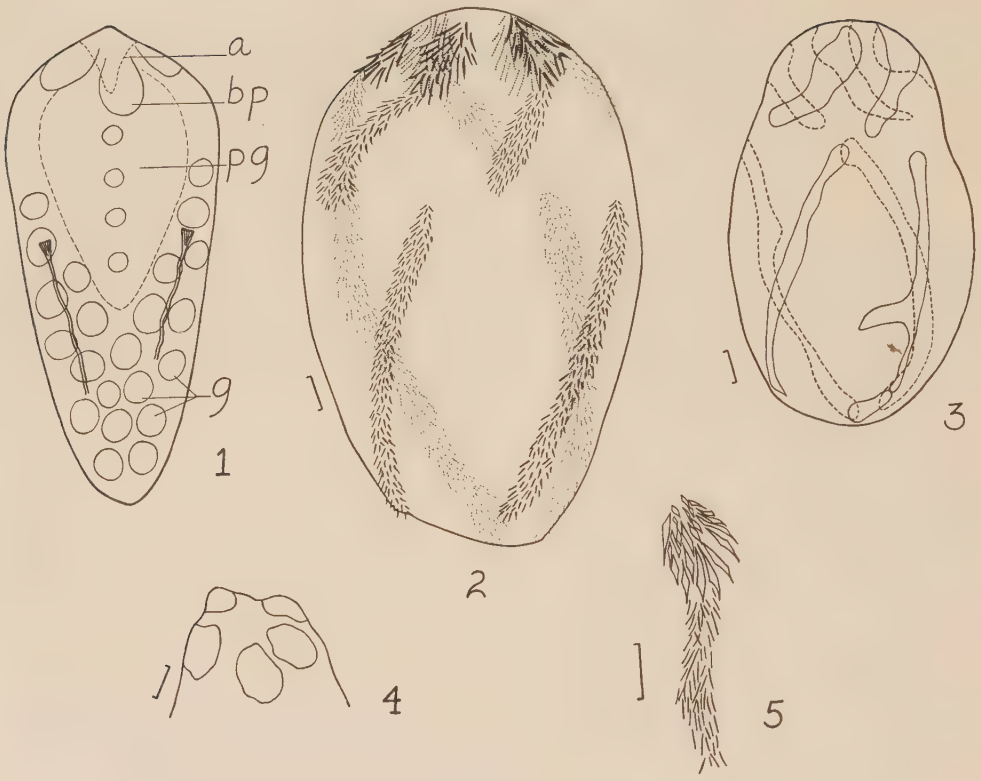
[1] Contribution from the Department of Zoology, University of Michigan.



EXPLANATION OF PLATE

Figures 2–5 drawn with the aid of the camera lucida. Scale—0.01 mm.

Figures 2, 4, 5 drawn from preparations stained with aceto-carmine and mounted in glycerine.

All figures of the miracidium of *Proterometra macrostoma*.

FIG. 1. Free-hand sketch of living miracidium. a—position of proboscis-like structure when withdrawn; bp—anterior large bristle plate; g—germinal tissue; pg—"primitive gut."

FIG. 2. Miracidium showing bristle plates.

FIG. 3. Outline showing dermal plates. Silver nitrate impregnation.

FIG. 4. Anterior end of miracidium. slightly oblique, showing relation of 5 anterior patches of large bristles.

FIG. 5. Detail of anterior patch of large bristles and its associated patch of small ones. Drawn bristle for bristle.

anterior bristles point backwards, this alternate extension and retraction of the anterior end is probably the method of penetration of the snail tissues.

DISCUSSION

In its lack of cilia and presence of bristle plates this miracidium is similar to those of *Azygia lucii* (Looss, 1894), *Otodistomum cestoides* (Manter, 1926) and *Ptychogonimus megastomus* (Willemoes-Suhm, 1871), also of the family AZYGIIDAE. *Proterometra macrostoma* makes the fourth genus of the AZYGIIDAE in which the miracidium is non-ciliated and has bristle plates. A similar non-ciliated, bristled miracidium has also been reported for three species of the genus *Halipegus* (family HEMIURIDAE), *H. ovocaudatus* (Creutzburg, 1890), *H. eccentricus* (Thomas, 1939) and *H. amherstensis* (Rankin, 1944).

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STUDIES ON FIVE NEW SPECIES OF XIPHIDIOCERCARIAE OF THE VIRGULA TYPE*

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INTRODUCTION

Although larval trematodes have been investigated to a considerable extent in the United States, knowledge of certain types has been limited almost entirely to species reported from other countries. One such type is a particular kind of xiphidiocercariae, the oral sucker of which possesses not only a stylet but also a peculiar structure, the so-called "Virgula organ." In his scheme of classifying cercariae, Lühe (1909) placed larvae of this type in a separate group, the Cercariae Virgulae, named for *Cercaria virgula* Filippi, one of the first of its kind to be described. *Cercaria geddesi* Ameel, 1939, appears to be the only larva of the Virgula type described in the United States, although a number of species have been reported from other regions.

Cable (1939) reported the occurrence of an unidentified Virgula cercaria in the prosobranch snail, *Goniobasis depygis* (Say), collected from McCormicks Creek, Indiana. Over a period of several years, these snails have been collected in large numbers to provide various types of larval trematodes for instructional purposes. It has been found that the Virgula species occurs in snails collected at all times of the year and causes a higher incidence of infection than does any other of the several species that may be present. Because of the apparent rarity of Virgula cercariae in the United States and the scarcity of information concerning their life cycles, the abundance and availability of the species from McCormicks Creek prompted an investigation of its morphology and life history.

Later, collections of prosobranch snails from other sources within a limited radius of Lafayette, Indiana, revealed the presence of four other distinctly different species of the Virgula type, thus providing material for a comparative study of five new species.

MATERIALS AND METHODS

The snails were collected from the various streams and placed in finger bowls. Those infected were isolated and kept alive as long as possible to provide cercariae for study. It was desirable to study larvae soon after emergence, since ageing ones showed numerous opacities and vacuolations that often obscured parts and hindered observation. Measurements and observations are from living specimens under light cover-glass pressure and either unstained or stained supravitally with neutral red. Approximate maximum measurements are those of the larvae fully extended, minimum measurements those of the most contracted specimens. All measurements are in millimeters and incidences of infection are based on emergence of cercariae from isolated snails.

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* The writer wishes to express his sincere thanks to Professor R. M. Cable for his guidance and encouragement throughout the present study, and to acknowledge the assistance of Miss Edna Banta in collecting snails, Dr. Henry van der Schalie who identified part of the molluscan material, and Miss Lois Kraus who provided infected snails found in connection with another study.

Prospective secondary intermediate hosts, chiefly May-fly naiads, were placed in dishes with infected snails for several days and examined at intervals for metacercariae, many of which could be detected in the living insect with a low-power binocular microscope. Infections were finally checked by dissecting the insects and removing metacercariae for study.

In the case of one species which readily penetrated and encysted in ephemerid naiads, metacercariae were permitted to develop until they were considered infective for the vertebrate host as determined by the appearance of the digestive ceca, clearing of the body and cessation of further development. Naiads containing these metacercariae were then fed forcibly to frogs, using a syringe-like glass tube and plunger. The frogs were maintained on cockroaches until killed later and examined for young worms.

OBSERVATIONS

Description of *Cercaria nyxetica* sp. nov. (Figs. 3–7)

Snail host: Goniobasis depygis (Say).
Locality: McCormicks Creek, Indiana.
Incidence: 5–50%, depending on the time of year.
Specific diagnosis: Distome xiphidiocercaria with characters of the Virgula group. Measurements of living cercariae are as follows: maximum length of body (extended) 0.2, minimum (contracted) 0.08; body averages 0.11 in length and 0.07 in width in slightly flattened specimens, neither greatly extended nor contracted. Tail 0.03 to 0.15 long depending on state of contraction, the moderately contracted tail measuring 0.07 long and 0.017 wide near the base. Oral sucker 0.04 in diameter; stylet 0.018–0.020 long and 0.006 wide at the base from dorsal (or ventral) aspect; Virgula organ 0.025 long and 0.04 wide at its anterior end. Diameter of pharynx 0.01, ventral sucker 0.02. Entire body spinose and provided with delicate setae set in small papillae which are more numerous anteriorly; tail with fine spines, those on the tip being slightly longer; contracted tail with deep marginal folds. Oral opening directed ventrad, oral cavity reduced by the large Virgula organ which is lighter in appearance than the sucker proper. Pharynx well developed; remainder of digestive system not apparent. Ventral sucker slightly posterior of middle of body. Genital primordium a C-shaped mass dorsal and slightly posterior to the ventral sucker. Three pairs of cephalic gland cells in posterior body half, with individual ducts opening near tip of stylet. Excretory vesicle U-shaped, wall lobate; tips of the cornua receive the main excretory tubules which extend forward, bifurcating slightly behind the level of the ventral sucker; complete flame cell pattern not determined. Develop in small, oval-round sporocysts, up to 0.3 in diameter.

The Virgula organ, which is the distinguishing feature of the group, may be discussed here, since its fate was traced farther in this species than in the others studied. In all of the species described in this paper, the organ appears to be a bilobed structure essentially in the form of a U with the two tapering cornua extending anteriorly, finally bending ventrally and converging to form a papilla. In larvae observed under light cover-glass pressure, this papilla can be seen to protrude into the oral opening. At its tip is a pore through which the secretion of the organ is expelled in droplets. Since the Virgula organ occupies the innermost part of the oral sucker, it has the appearance of a structure simply crowded into the oral cavity and pressed against the wall of the sucker without being actually embedded in it. In fact, it is difficult to say whether the inner membrane of the sucker does enclose the organ; if not, then it adheres very closely to the muscular portion of the sucker.

The Virgula organ is filled with a homogeneous substance which is oily in appearance and stains an orange or salmon color with neutral red. After discharge from the organ, this substance becomes granular, apparently due to its emulsification with water.

The function of the Virgula organ is obscure. Sewell (1922) suggested that it might serve as a support for the oral sucker during penetration of the second intermediate host. It seems, however, that this function would be a minor one in species which have diminutive Virgula organs. Furthermore, the discharge of the organ's contents as described above indicates a glandular function. This does not seem to be essential to penetration of the intermediate host, however, since the organ persists beyond penetration and even encystment and can be seen in young metacercariae.

The stylet (Fig. 4) is embedded in the sucker with the tip exposed and directed antero-ventrad. At about one-fourth its length from the tip, it bears a pair of dorsolateral, knob-like prominences or shoulders.

The anteriormost pair of cephalic glands is on a level with the ventral sucker. It and the pair immediately following are similar in appearance, with finely granular contents and ducts which open near the tip of the stylet. The posterior pair of cephalic glands is coarsely granular and stains darker with neutral red than do the others. Also, the ducts of the posterior pair open distinctly nearer the mouth.

The cercaria is very active. It swims with the body flexed ventrally and tail lashing rapidly. When not swimming, it creeps over the substratum in an inchworm manner, using its suckers alternately.

The sporocysts (Fig. 7) occur in large numbers in the digestive gland of the snail. They are small, oval-round and colorless, and often contain about a dozen cercariae.

There is considerable seasonal variation in the incidence of infection. The greatest number of snails are infected during the late summer and autumn when the incidence was found to be close to fifty per cent. In spring and early summer, however, only five to ten per cent of the snails harbored the parasite.

Notes on the Life History of Cercaria nyxetica

Cercaria nyxetica has been found to penetrate and encyst in aquatic insect naiads of the orders Odonata and Ephemeroptera. The exact path of entry into these second intermediate hosts has not been determined. Metacercariae are widely distributed in the infected naiad, occurring throughout the abdomen, thorax and frequently in the femoral muscles of the legs. Encystment usually does not occur immediately and may be delayed as long as two days during which the larvae may be seen moving actively in the muscles of the leg and thorax or in the fatty tissue of the abdomen. The stylet is shed during or shortly after encystment, but persists and can be seen embedded in the cyst wall or partly disintegrated in the cystic fluid of even the oldest metacercariae. Under laboratory conditions at room temperature, the metacercariae develop in five weeks to the stage shown in Fig. 6, and attain a diameter of 0.18 to 0.25. At this time, the body begins to clear of the refractile globules that appeared soon after encystment and rendered it opaque. As the metacercariae age, the penetration glands and Virgula organ disappear and the digestive system becomes distinct. A very short prepharynx is present and the intestinal ceca end at a level with or slightly anterior to the ventral sucker. The excretory bladder promises to be U-shaped in the adult and is filled with highly refractile granules. Other details of internal structure were not distinct.

For experimental work, May-fly naiads were used, because their small size and

transparency made it possible to detect metacercariae through the body wall when the live insect was examined with the dissecting microscope. High mortality of infected naiads, however, greatly limited attempts to complete the life history.

The only complete life history reported for the Virgula group is that of *Loxogenes liberum,* traced by Okabe (1937). Since species of *Loxogenes* occur in frogs, it seemed advisable to test them as possible definitive hosts for the adult of *C. nyxetica.* First, an attempt was made early in the summer of 1944 to determine whether frogs collected in the vicinity of the snail host harbored an adult trematode that might be the one in question. Frogs were rare in the stream, only one being captured after careful search. Examination of this frog revealed the presence of a single, large specimen of *Loxogenes bicolor* Krull in an hepaticoduodenal cyst. An attempt was then made to determine whether *C. nyxetica* might be the larva of *L. bicolor* by feeding infected May-fly naiads to two frogs of a lot obtained from a commercial source outside the state. Several of the lot had been used previously for other purposes and found to be free of species of *Loxogenes.*

One of the experimental frogs was killed and examined five days after being fed naiads containing 40-day-old metacercariae and was found to be negative for any sort of trematode. The same results were obtained when the second frog was sacrificed several days later. Scarcity of materials and transportation difficulties prevented further attempts to infect frogs during the season of 1944.

There are few snails of any sort in McCormicks Creek except *Goniobasis depygis,* the host of *C. nyxetica.* The creek is rocky, small, and rapid with few pools of any considerable depth and thorough collecting is easily accomplished. Thousands of *G. depygis* and as many other snails as could be found have been collected from McCormicks Creek over a period of nine years and only one species of Virgula type cercariae has ever been discovered from that locality. It therefore seems likely that this species may be the larva of *L. bicolor* which certainly is known to occur in frogs of the vicinity, even though feeding experiments were negative. Since only two frogs were used and these were in poor condition because of prolonged captivity, the results obtained are considered to be inconclusive.

Description of *Cercaria neustica* sp. nov. (Figs. 10 and 11)

Snail host: Pleurocera acuta Rafinesque.
Locality: Eel River, North Manchester, Indiana.
Incidence: 1.0%.
Specific diagnosis: Maximal body length extended 0.23, minimum length contracted 0.08; body of moderately contracted specimens under light pressure averages 0.116 long, 0.065 wide, and 0.058 thick. Moderately contracted tail 0.07 long and 0.02 wide at the base. Oral sucker 0.03 in diameter, with relatively small Virgula organ 0.019 wide at the anterior end and 0.014 long. Stylet averages 0.023 long by 0.008 wide at the base, tip curved ventrally. Pharynx 0.012 long by 0.009 wide, esophagus and intestine not observed. Ventral sucker 0.019 in diameter and situated at approximately the middle of the body. Genital primordium 0.020 wide by 0.022 long, C-shaped and located at or slightly posterior to level of ventral sucker. Three pairs of cephalic glands, the most posterior having the coarsest cytoplasmic granules. Ducts of anterior pair separate from others, bending dorsad and then mediad before passing over the oral sucker. Flame cell pattern 2 [(2+2+2) + (2+2+2)]. Excretory bladder U-shaped and 0.023 long when fully distended. Develop in oval-round sporocysts in the digestive gland of the mollusk. No cysts were found in May-fly naiads after exposure for two weeks to a large number of cercariae.

Of the species described here, *C. neustica* was the only one in which the flame cell pattern could be seen with any degree of clarity. The main collecting tubule

of each side extends anteriorly a short distance and divides before reaching the ventral sucker to form a posterior and an anterior secondary tubule. Each secondary tubule receives the capillaries of six flame cells in groups of two, one dorsal and one ventral. The collecting tubules are seen clearly at rare intervals and, because the body is quite thick, the capillary connections of dorsal and ventral flame cells of each group are observed accurately only from the lateral aspect. The excretory system was obscured also by the refractile edges of what are presumably cystogenous glands just beneath the cuticle.

To the writer's knowledge, the complete flame cell pattern has been determined in only two other Virgula cercariae, viz., *C. helvetica* IX Dubois (1928) and *C. virguloides* Porter (1938). The pattern in the former species is apparently the same as that of *C. neustica,* and is one that has been described for several xiphidiocercariae of other types. The pattern described for *C. virguloides,* however, consisted of but eight pairs of flame cells arranged with two pairs on each of the anterior and posterior collecting tubules.

As in the case of the other species studied, the two anteromost pairs of cephalic glands always discharge their contents before the posterior pair. The ducts of the posterior pair are very slender and follow a more or less tortuous path along the ducts of the second pair. In most specimens, the cytoplasm of the posterior pair stained more intensely with neutral red than did the others. Sometimes, however, all of the glands appeared the same color.

Description of *Cercaria meringura* sp. nov. (Figs. 1 and 2)

Snail host: Goniobasis livescens (Menke).
Locality: Tippecanoe River, Battle Ground, Indiana.
Incidence: 1.24%.

Specific diagnosis: Body length extended 0.22, contracted 0.1; moderately contracted body 0.13 long and 0.09 wide at level of ventral sucker. Tail extended approximately 0.16, contracted 0.045. Oral sucker 0.049 in diameter; stylet 0.046 long, 0.008 wide at base. Virgula 0.030 in diameter. Body spinose with more conspicuous spines around opening of ventral sucker. Tail aspinose except at tip which bears a patch of long spines. Body with papillae bearing fine setae as in other species reported here. Pharynx 0.015 long and 0.02 wide, often obscured by a mass resembling oil droplets; remainder of digestive system not observed. Genital primordium posterior to the ventral sucker. Three pairs of cephalic glands and ducts of each side passing forward in a single bundle and opening separately in a row close to stylet tip; anterior ends of ducts more distinct than in other species studied. Small bodies with the staining characteristics of the cephalic glands occur consistently in two pairs near the pharyngeal level and additional ones are sometimes present. Develop in oval-round or slightly irregular sporocysts in the digestive gland of the snail. Sporocysts measure up to 0.25 long and contain about a dozen cercariae. Attempts to induce penetration and encystment in May-fly naiads were unsuccessful.

Description of *Cercaria nothrica* sp. nov. (Figs. 8 and 9)

Snail host: Pleurocera acuta Rafinesque.
Locality: Tippecanoe River, Battle Ground, Indiana.
Incidence: 0.51%.

Specific diagnosis: Length of body extended 0.173, contracted 0.077; moderately contracted body 0.092 long and 0.073 wide at level of ventral sucker. Tail extended approximately 0.115 long, contracted 0.023 long and 0.023 wide at the base. Oral sucker 0.043 wide; stylet 0.022 long and 0.005 wide at base, tip curved ventrally. Virgula organ 0.030 long and 0.038 wide at the anterior end. Ventral sucker 0.020 wide, situated in a prominent bulge of the ventral surface. Body and tail spinose, longer spines on tail tip. Papillae with fine setae scattered over body. Pharynx 0.01 long; remainder of digestive system not observed. Genital primordium posterior to ventral sucker. Three pairs cephalic glands, the anterior pair with coarser granules than the others; neutral red stains posterior pair darkest. Cephalic gland ducts of each side pass anteriorly as a single bundle with separate openings near tip of stylet. Bladder U-shaped. Develop in oval-round sporocysts up to 0.16 long in digestive gland of snail.

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EXPLANATION OF PLATE I

All figures of entire cercariae are drawn to the same scale and stylets are represented at twice that magnification.

FIG. 1. *Cercaria meringura*, ventral view.
FIG. 2. Stylet of *C. meringura*, dorsal view.
FIG. 3. *Cercaria nyxetica*, ventral view.
FIG. 4. Stylet of *C. nyxetica*, dorsal view.
FIG. 5. Young metacercaria of *C. nyxetica*.
FIG. 6. Fully developed metacercaria of *C. nyxetica*.
FIG. 7. Sporocyst of *C. nyxetica*.

EXPLANATION OF PLATE II

All figures of entire cercariae are drawn to the same scale and stylets are represented at twice that magnification.

FIG. 8. *Cercaria nothrica*, ventral view.
FIG. 9. Stylet of *C. northrica* in (a) lateral and (b) dorsal aspects.
FIG. 10. *Cercaria neustica*, ventral view.
FIG. 11. Stylet of *C. neustica* in (a) lateral and (b) dorsal aspects.
FIG. 12. *Cercaria tranoglandis*, ventral view.
FIG. 13. Stylet of *C. tranoglandis* in (a) dorsal and (b) lateral aspects.

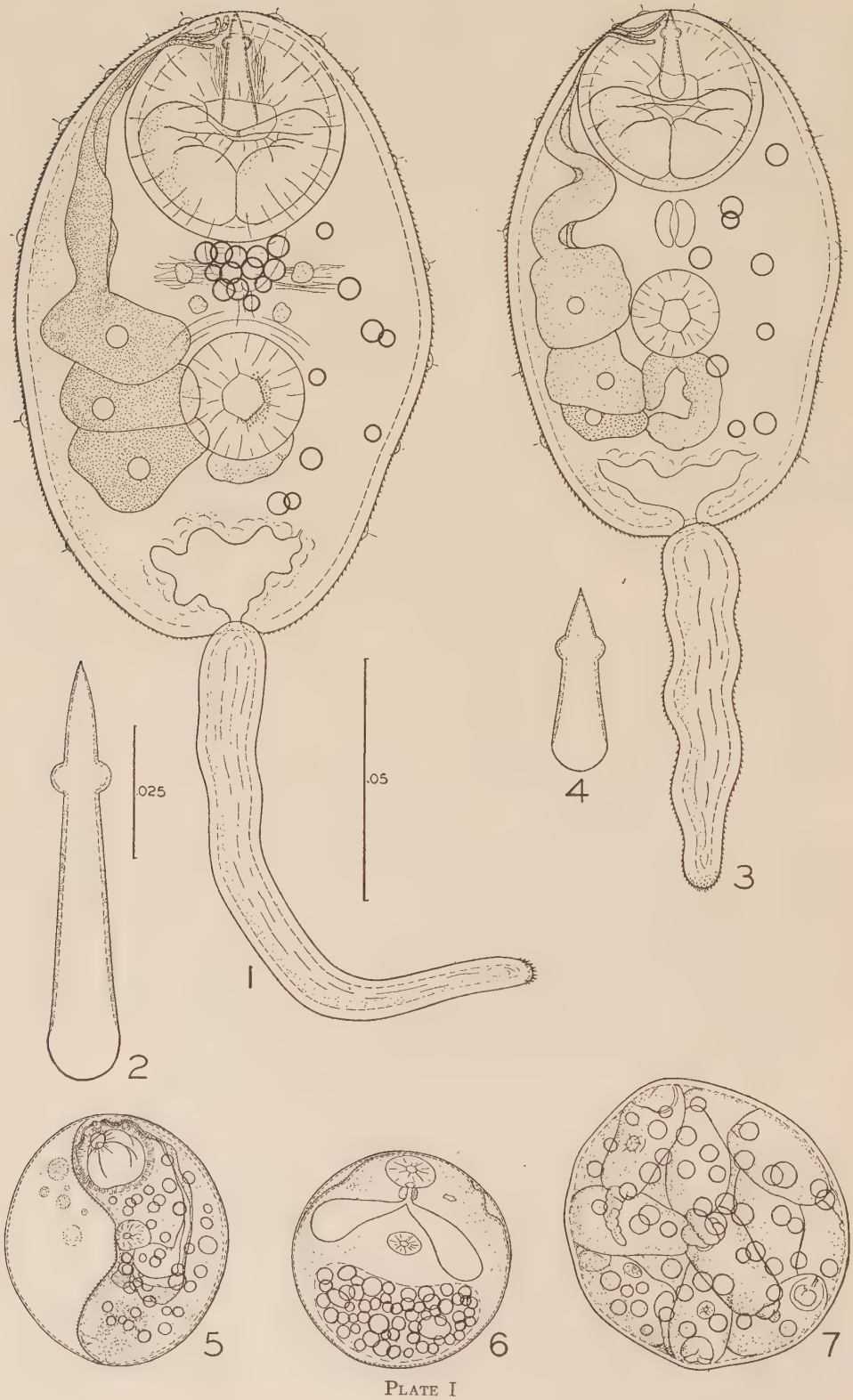

PLATE I

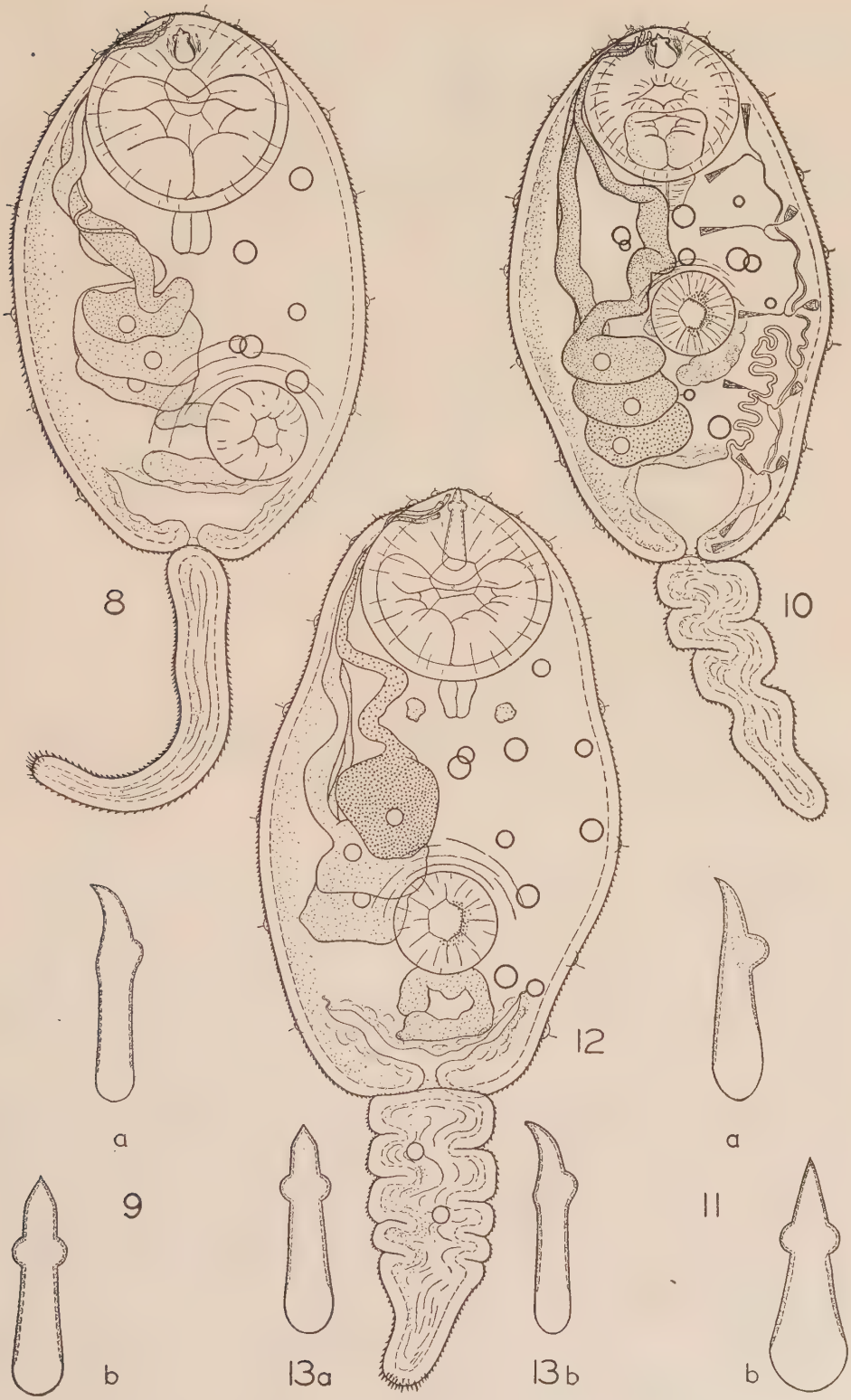

PLATE II

TROMBICULA FRITTSI N. SP. (ACARINIDA: TROMBICULIDAE)

LIEUT. (JG) G. W. WHARTON, H(S), USNR[1]
U. S. Naval Medical Research Unit No. 2[2]

Specimens of a new species of *Trombicula* were collected on Bougainville Island by J. M. Fritts, PhM2c, during the summer and fall of 1944. These specimens are described under the name *Trombicula frittsi.*

Womersley and Heaslip (1943) devised a series of standard measurements of the scutum. Their system is adopted and the designations of the measurements follow:

AW—distance between the bases of the anterior-lateral setae.
PW—distance between the bases of the posterior-lateral setae.
SB—distance between the centers of the pseudostigmata.
ASB—greatest perpendicular distance from a line drawn through the centers of the pseudostigmata to the anterior margin of the scutum.
PSB—greatest perpendicular distance from a line drawn through the centers of the pseudostigmata to the posterior margin of the scutum.
AP—distance between the bases of the anterior-lateral and posterior-lateral setae.
AM—length of the anterior-median seta.
AL—length of the anterior-lateral setae.
PL—length of the posterior-lateral setae.
S—length of the pseudostigmatic organs or sensillae.
DS—length of the dorsal setae.

All of the measurements are given in microns. While consideration of these standard data are extremely useful, careful allowances must be made for variations within species and unavoidable errors in measurement.

DESCRIPTION

Trombicula frittsi n. sp. (Figure 1). Only engorged larvae were collected. Length including capitulum, 400 microns. Maximum width, 250 microns. Color red. Striae on cuticle not prominent.

Palps (Figure 1, C and D): Segment 1 with a branched seta originating from the anterior-lateral region. Segment 2 rounded laterally, seta with not more than two delicate branches. Segment 3 with a nude seta. Segment 4 with nude dorsal and lateral setae, ventral seta with two lateral branches. Segment 5 with one proximal-ventral sensory seta, one medial-ventral branched seta, one central-ventral nude seta, one lateral nude seta, and two heavily barbed apical setae. Palpal claw trifurcate. Cheliceral shield well developed with one pair of nude setae.

Chelicerae (Figure 1, C and D): Basal segment evenly rounded, 20 microns long, 15 microns wide, and lightly ornamented with small pits. Distal chitinous segment strongly curved with a single recurved dorsal tooth and a single recurved ventral tooth. Apex sharp dorsally, rounded ventrally.

Legs (Figure 1, A and B): Coxae each with a single branched seta. One sensory seta on each tarsus. Tibia III with a sensory seta.

Scutum (Figure 1, E): Pentagonal with a few scattered pits. Anterior angles smallest, posterior-median angle greatest. Posterior margin folded at its apex. Setae all with short barbs. Pseudostigmatic organs filiform with short lateral branches on the distal two-thirds. Standard measurements in microns:
AW—43, PW—56, SB—17, ASB—17, PSB—21, AP—18, AM—22, AL—21, PL—29, S—39, DS—22-26.

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[1] On military leave from Duke University.

[2] The opinions expressed are the author's and not necessarily those of the Navy Department.

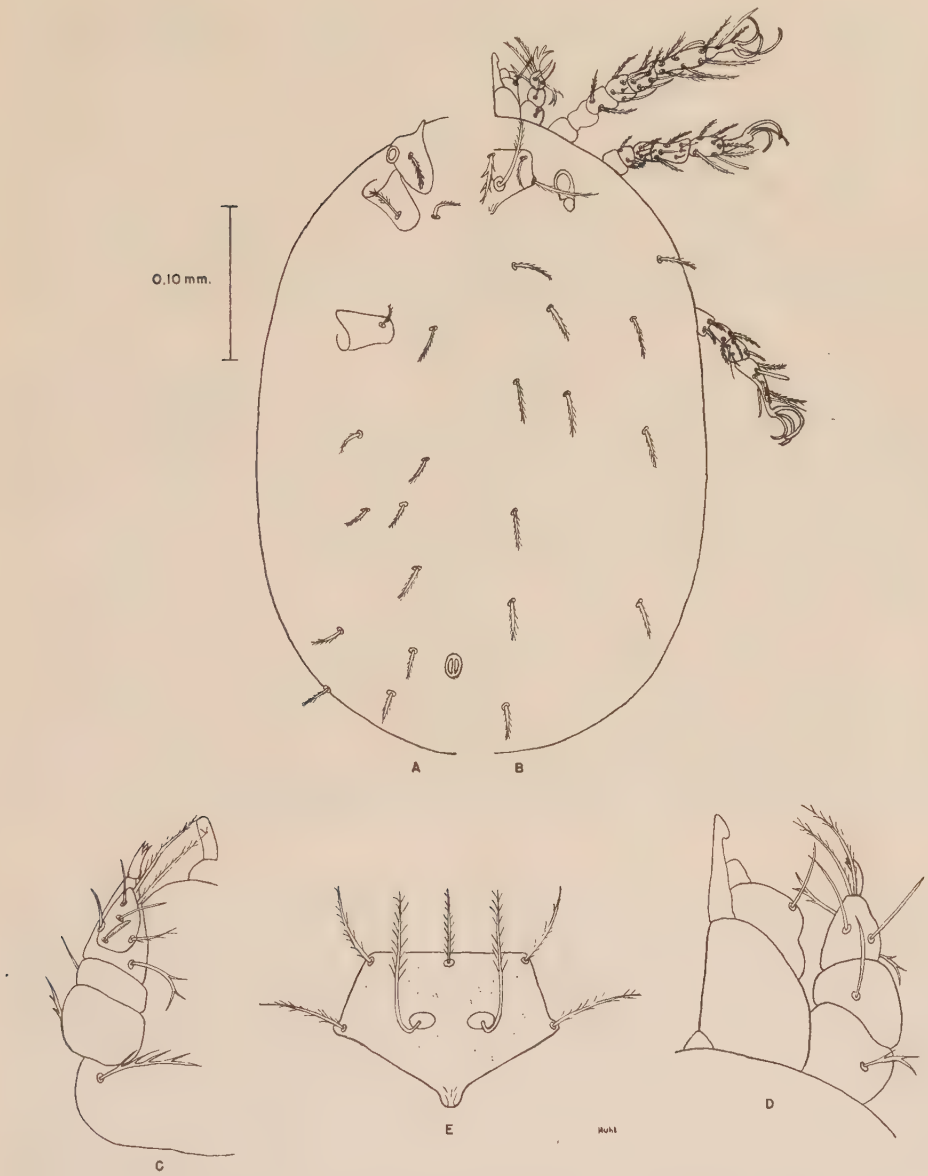

FIG. 1. *Trombicula frittsi* n. sp. A, ventral view; B, dorsal view; C, ventral view of capitulum × 1000; D, dorsal view of capitulum × 1000; E, scutum × 500.

Setae: Dorsal formula, 2–6-6-4-2-2–; ventral formula, 2–2–4–4–4–4–2. Dorsal and ventral setae similar in shape, all with short lateral barbs.

Hosts: Gehyra oceanica, Varanus indicus, and *Rattus praetor.*

Locality: Cape Torokina, Empress Augusta Bay, Bougainville Island, Australian Mandate.

Type: U. S. National Museum.

Paratypes: U. S. National Museum; South Australian Museum.

Diagnosis:—Trombicula frittsi can be readily distinguished from all other members of the genus by the ridges and folds on the scutum at its posterior-median angle.

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TRICHURIS SPECIES FROM CALIFORNIA RODENTS

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Study of a collection of whipworms obtained from California rodents collected by the Hastings Natural History Reservation near Monterey, California, revealed the presence of four species, three of which are new, and indicates a marked host specificity for species of this genus. Descriptions of the new species follow, and also some additions to Hall's (1916) description of *T. fossor*.

Trichuris fossor Hall, 1916

Specimens from *Thomomys bottae bottae* are larger than those described by Hall, 1916, from *Thomomys fossor* in Colorado, the males measuring 28 to 30 mm in length, and the females 28 to 33 mm. In Hall's specimens, which were probably immature, the anterior portion of the body exceeded the posterior portion in length, but in my mature specimens the posterior portion equals or exceeds the anterior portion in length. The specimens agree in the bilobed end of the male body, the bell-shaped, spiny, spicular sheath, and the length of the spicule (about 1.5 mm long in my specimens). The spicule flares slightly a short distance behind the tip. The cloacal tube is about 2.5 mm long, and is joined by the spicular tube near the middle of its length. The ejaculatory duct joins the vas deferens at about the middle of the length of the posterior portion of the body. In the female the vulva is not situated on a prominence, and the ovejector is from 1.1 to 2 mm long, gradually widening into the uterus. The anus is subterminal. The eggs, not seen by Hall, are relatively broad, measuring 64 to 68 microns by 35 to 38 microns.

Trichuris citelli, n. sp.

Description: Male 34.5 to 38.5 mm long; posterior part of body 12.5 to 14.5 mm long, anterior part 21.5 to 24.5 mm long, the ratio being 3:5 to 4:7. Maximum diameter of posterior part about 600 microns; diameter at end of esophagus about 350 microns; diameter of anterior part of body gradually increasing from 75 to 200 microns. Posterior end of body bilobed. Spicule 1.3 to 2.1 mm long, tapering in its proximal two-thirds from a diameter of 75 to 100 microns to about 45 microns, then maintaining this diameter to about 60 microns of distal end, where it abruptly narrows to a conical tip. Spicule sheath, when fully evaginated, projecting from body 270 to 360 microns, the exserted part forming a bulb about 300 microns broad, with the spines developed best on its proximal surface. Cloacal tube (cloaca to junction of ejaculatory duct and intestine) 1.5 to 1.9 mm long, spicular tube joining it about 1 to 1.3 mm from posterior end. Junction of ejaculatory duct and vas deferens 6.5 to 8.5 mm from posterior end of body. Vas deferens 5 to 6 mm long, looping back to form testis just behind end of esophagus. Testis lobulated, lobes 800 to 900 microns long posteriorly, 150 to 450 microns long near middle and anteriorly. Testis ends about at proximal end of cloacal tube.

Female about 45 mm long; posterior part of body 18 to 19.5 mm long, anterior part 26 to 27.5 mm long, the ratio being 2:3 to 3:4. Vulva situated on a prominence, approximately at end of esophagus. Maximum diameter of posterior part of body 700 microns, of anterior portion increasing from 100 to 200 microns; diameter at vulva 350 to 400 microns. Ovejector 1.3 to 1.6 mm long, enlarged into a chamber at posterior end. Uterus loops back to form ovary about 1.5 to 2 mm from posterior end. Rectum 225 to 275 microns long. Eggs 70 to 74 microns by 33 to 35 microns.

Location: Caecum.
Host: Citellus beecheyi.
Locality: Hastings Natural History Reservation, California.
Type and allotype: U. S. National Museum, Helm. Coll. Nos. 45,824, 45,825.

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This species resembles *T. muris* (Schrank, 1788) Hall, 1916, as redescribed by Cerecero, 1943, in many respects, but is much larger, with a much longer and stouter spicule and larger eggs. In *T. muris* the proximal end of the cloacal tube is about one-third the distance from the posterior end of the body to the junction of the ejaculatory duct and vas deferens, whereas in *T. citelli* it is about one-fourth of this distance.

Trichuris perognathi

Description: Male 25 to 30 mm long, posterior part of body approximately same length as esophageal part. Maximum diameter of thick part about 350 microns, of esophageal part about 90 microns. Spicule 0.9 to 1.15 mm long, 20 microns in diameter near the rounded tip, flaring to diameter of about 45 microns about 250 to 300 microns from tip, then narrowing somewhat, and broadening again at proximal end to diameter of about 50 microns. Spicule sheath bell-shaped when exserted, covered with dense, blunt spines on terminal 220 to 300 microns. Spicular tube joins cloacal tube about 400 microns from posterior end. Cloacal tube about 2 mm long. Junction of ejaculatory duct and vas deferens not observed. Testes irregularly lobulated.

Female 46 to 47 mm long, the esophageal portion equal to or slightly shorter than thick portion of body (21 to 23 mm long). Maximum diameter about 550 microns, near middle of thick part of body. Body tapers markedly posteriorly, and ends in a truncated tip about 150 microns in diameter. Vulva situated just posterior to end of esophagus, and marked by a backward projecting cuticular evagination about 100 microns long. Ovejector about 0.8 to 1.1 mm long, widening abruptly into the uterus. Eggs narrow, 65 to 67 microns by 31 to 33 microns.

Location: Caecum.

Host: Perognathus californicus californicus.

Locality: Hastings Natural History Reservation, California.

Type and allotype: U. S. National Museum, Helm. Coll. Nos. 45,828, 45,829.

This species is readily distinguished by the backward projecting cuticular evagination on which the vulva is situated, by the tapering of the posterior end of the body of the female, by the failure of the esophageal portion of the body to exceed the posterior portion in length in either sex, and by the characters of the spicule and its sheath in the male.

Trichuris neotomae

Description: Male 22 to 23 mm long, posterior part of body 7.5 to 8 mm long, anterior part 14 to 17 mm long, the ratio being 1 : 2 to 3 : 5. Maximum diameter of posterior part 475 to 500 microns, diameter of anterior part about 140 microns proximally, tapering to about 125 microns in cephalic half. End of body distinctly bilobed, the indentation between the lobes being about 50 to 60 microns deep. Spicule 1.15 to 1.23 mm long and 30 microns wide near tip, flaring to about 45 microns about 0.5 mm from proximal end. Spicular sheath, when fully evaginated, protrudes from body about 440 microns, the protruded part with an expanded tip 137 microns broad and 155 microns long, then narrowing to a diameter of 65 microns, the entire extruded part covered with spines. Inside body, cloacal tube heavily chitinized and corrugated for about 265 microns, then widening to a still more heavily chitinized but less markedly corrugated chamber 200 to 250 microns long and 100 microns wide, ending at point where spicular tube and cloacal tube join, about 570 to 600 microns from posterior end of body. Rest of spicular tube with thin walls. Junction of spicular tube and cloacal tube to proximal end of cloacal tube about 660 to 700 microns, entire cloacal tube being, therefore, about 1.2 to 1.25 mm long. Ejaculatory duct about 3 mm long, and vas deferens about 3.75 mm long, the two joined by short, narrow duct. Testis irregularly lobulated, extending from posterior end of esophagus to proximal end of cloacal tube.

Female 28 to 34 mm long; posterior part of body 13 to 16 mm long, anterior part 16 to 21 mm long, the vulva at posterior end of esophagus, dividing body approximately 1 : 1.25 to 1.6. Vulva situated on a slight prominence. Maximum diameter of posterior part of body 700 to 800 microns, of anterior part 150 to 180 microns. Ovejector about 1 to 1.4 mm long, with space for only a single row of eggs. Anus 30 to 40 microns from posterior end, which is bluntly rounded. Eggs long and narrow, measuring about 90 by 40 microns.

Location: Caecum.

Host: Neotoma fuscipes.

Locality: Hastings Natural History Reservation, California.

Type and allotype: U. S. National Museum, Helm. Coll. Nos. 45,826, 45,827.

This species resembles *T. muris* as described by Cerecero (1943) in most respects, but differs in the greater length and stoutness of the spicule, the longer ovejector, and the much larger eggs. It is most strikingly characterized by the heavily chitinized and corrugated walls of the cloacal tube distal to the junction of the spicular tube.

SUMMARY

Trichuris fossor is reported from a new host, *Thomomys bottae bottae,* and three new species of *Trichuris* are described: *T. citelli* from *citellus beecheyi, T. neotomae* from *Neotoma fuscipes,* and *T. perognathi* from *Perognathus californicus californicus.*

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HELMINTHS FROM THE BOB-WHITE QUAIL IN TEXAS

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From 1941 to 1943, in cooperation with several men of the Texas Game, Fish and Oyster Commission, we examined 290 Texas Bob-white Quail (*Colinus virginianus texanus*) for helminth parasites, under the supervision of Dr. Asa C. Chandler. The majority of the examinations was made by the junior author; the senior author is responsible for the identifications. Actually, only the viscera were examined of all of the 290 birds; also tracheae of 55 and entire bodies of 12 were available. The quail were taken by Valgene W. Lehmann, W. Garner Fuller, Rollin H. Baker, J. M. Carlisle, and the senior writer. 276 birds came from South Texas (Jim Hogg, Jim Wells, Duval, and Zapata Counties) and 14 from Central Texas (Coleman, Harris, Trinity, and Colorado Counties). All of the birds were full-grown except for six downy chicks. It is suggested that comparison be made with the work of Cram, Jones, and Allen (1936).

CESTODES FOUND

Raillietina (*Raillietina*) *colinia* Webster, 1944, was encountered only once (Webster, 1944), in September from Central Texas.

Raillietina (*Skrjabinia*) *cesticillus* (Molin, 1858) Joyeux, 1923, was taken only once (Webster, 1944), a heavy infestation mixed with the preceding species.

Paricterotaenia sp., was encountered once—a single worm from South Texas in December.

Rhabdometra odiosa (Leidy, 1887) Jones, 1929, was found in 14 quail from South Texas, not more than seven worms in any one infestation. There was 19% infestation in 47 quail taken in South Texas from May to August, but only 2% infestation in 205 birds taken there in December and January.

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NEMATODES FOUND

Aulonocephalus lindquisti Chandler, 1935, has previously been reported only in the original description (Chandler, 1935), which was based on specimens from a Scaled Quail (*Callipepla squamata*) taken at Uvalde, only 150 miles northwest of the origin of most of the present records. This nematode has a geographical correlation; of 14 quail examined from Central Texas there was only one record, as compared with 92% infestation of the 270 adult quail from South Texas. The worms were commonest in the caeca although they frequently occurred in the large intestine; mature males varied from 8 to 10.6 mm in length; mature females varied from 10 to 14.5 mm in length. One difference in anatomy was found as compared with Chandler's description: position of the vulva was found to be slightly anterior to the middle of the body, dividing the body in a ratio of from 10: 11 to 10: 13 (fifteen females measured, from fifteen hosts), whereas Chandler reported that the vulva in his three female specimens was at, or slightly posterior to, the middle of the body. As many as 300 worms were taken from a single host, but 15 to 40 was most common.

Cyrnea sp. occurred but once; two males were taken from a proventricular lining in June from South Texas.

Syngamus trachea (Montagu, 1811) Chapin, 1925, was taken twice, in summer from South Texas.

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RESEARCH NOTES

PINWORM INFESTATION AMONG CHILDREN OF RURAL COMMUNITIES

During the winter of 1942–43, 315 school and preschool children, residents of several small towns in southwestern South Dakota, were examined for pinworm ova. The method used was that described by Jacobs (1942, Journal Pediatrics **21**: 497), i.e., a piece of Scotch tape held over a tongue depressor with adhesive side out was applied to the perianal folds, flattened on a glass slide, and examined microscopically. Each child who was examined had to submit a filled-in questionnaire which included a written request from his parent. The parent was asked to state (yes or no when possible) whether the child slept well, ate well, was "nervous" in his opinion, was bothered by rectal itching, had many stomachaches or similar pains. Each child was weighed and measured at time of examination. With occasional exceptions, each child was examined but once.

Of the 315 children, 124 or 39.4% were positive for pinworm ova. This percentage would doubtless have been higher had repeated examinations been made. As it is, it compares closely with reported incidences among urban and institutionalized children. 36.2% of the boys who were examined (59 of 163), and 42.8% of the girls (65 of 152), were positive. The distribution of infection according to ages was as follows:

Age at last birthday	No. pos.		No. examined		% pos.	
Pre-school: 1 through 5	11		44		25	
School:						
6	20		36		56	
7	16		37		43	
8	12		40		30	
9	19		37		43	
10	22	113	46	271	48	41
11	9		36		25	
12	13		24		54	
13 through 15	2		15		13	
Total	124		315		39.4	

A sharp rise in rate of infestation was apparently coincident with attendance at school.

Information elicited from the parents showed the following differences—or similarities—between children found to be infected and children who upon one examination were negative for pinworm ova:

	Among 191 "negative" children	*Among 124 "positive" children*
No complaints	45%	35%
Complaints:		
1) anal pruritus	15%	27%
2) "nervousness"	36%	42%
3) underweight	17%	15%
4) insomnia	17%	9%
5) anorexia	15%	16%

Since the true incidence of infestation was not determined, undue significance cannot be attached to these figures. They suggest, however, that the frequent occurrence of symptoms among infested children should not be assumed to be associated with the infestation unless comparison with a similar uninfested group shows that the assumption is warranted.—Evelyn A. Mauss, *Division of Public Health Laboratories, State Board of Health, Rapid City, South Dakota*) in cooperation with the Fall River-Custer Counties Health Dept. and with the school authorities of those counties.)

CHILLING AS A MEANS OF RETAINING THE VIABILITY OF THE SPOROZOITES OF *PLASMODIUM GALLINACEUM*

In sporozite-induced *Plasmodium gallinaceum* infections the procedure of exposing chicks to the bites of single infected *Aedes aegypti* mosquitoes is time-consuming and results in infections in only 85 per cent of the birds according to Coatney et al (1945 Am. J. Hyg. **41**: 109). Later, Coatney et al (1945 Am. J. Hyg. **41**: 119), reported infections in only 97.5 per cent of

200 birds into which infected isolated salivary glands had been injected subcutaneously. For many purposes, for example drug testing, it is desirable to avoid any uninfected birds in an experiment. It has been our experience, as well as that of others (Coatney et al 1945, Am. J. Hyg. **41**: 119; personal communication with R. J. Porter) that the injection of pooled sporozite suspensions invariably results in an infection.

Needless to say, it is also desirable to have uniform infections throughout an experiment. Since the viability of sporozoite suspensions may be adversely affected by unfavorable conditions, attempts have been made to improve the preparation and use of these suspensions. To reduce the time required for the preparation of the infective material, whole mosquitoes (etherized less than 1 minute) are ground in a Ten Broeck tissue grinder (Scientific Glass Co.) for less than 5 minutes. Porter (1942, J. Inf. Dis. **71**: 1) indicated suspensions prepared in normal saline were irregularly infective, therefore normal chicken serum was used. To remove larger particles of chitin, etc., the material is strained three times through several layers of gauze. In each experiment, two control groups of 5 birds each are used; the first control group inoculated at the beginning and the second at the end of a series of test groups.

When kept at room temperature the viability of the sporozoites decreases during the period of about an hour required to inoculate intravenously 200 to 300 chickens. This is evidenced by the significant difference in parasitemia on the eighth day of infection. For example, in four separate tests the average parasitemia of the terminal control group in a series of 200 birds expressed as a percentage was only 10, 23, 23 and 11.6 per cent of the average parasitemia of the initial control group. Dr. E. Waletzky of this laboratory suggested that sporozoites might retain their viability to a greater extent if the ground mosquito material was chilled as soon as possible and kept in an ice bath during the period of inoculation. When such a procedure was used a more uniform infection was obtained since corresponding percentages for five separate trials were as follows: 71, 52, 77, 63 and 91.

The sparing effect of low temperature is also indicated by the increased virulence of chilled preparations. In 11 separate tests using an inoculum per bird of the unchilled sporozoites from about one mosquito, the average parasitemia on the eighth day of the infection for the first control group varied from 3 per cent to 48 per cent with a mean of 22.17 and a standard deviation of 14.1. The first trial using about the same size inoculum of chilled sporozoites resulted in the death of 3 of the 5 birds in the first group on the seventh day of the infection and parasitemias in the remaining two birds of 38 and 68 per cent. The second trial resulted in the death of all 5 birds on or slightly before the seventh day of the infection. Because of the apparent increase in the virulence of the sporozoites only about one-half mosquito was used for each inoculum in the third trial. This dose resulted in an average parasitemia of 44 per cent on the seventh day. In five subsequent trials the inoculum was reduced to consist of sporozoites of about one-tenth of a mosquito. The mean parasitemia of 4 of the first control groups was 36, 34, 34 and 13 per cent. In the fifth test 3 of the 5 birds died on the seventh day and the parasitemia of the remaining 2 was 45 and 51 per cent on the eighth day. Oöcyst counts indicate that the increased virulence seen following the adoption of the procedure of chilling the sporozoites cannot be accounted for by any significant increase in the intensity of the infections in the mosquitoes. This increase in virulence of the standard inoculum due to chilling was unexpected and suggests that even as short an exposure as 15 minutes (the maximum time intervening between the preparation and use of an inoculum) to room temperature results in marked injury to sporozoites.

Summary: Chilling pooled sporozoite material results in more uniform infections throughout a series of animals in the same experiment.—Sterling Brackett and Carrie Ola Hughes, *Stamford Research Laboratories of The American Cyanamid Company, Stamford, Conn.*

CONFLICTING VIEWS IN REGARD TO *IODAMOEBA WILLIAMSI*

It is frequently stated in text-books, that cysts of *Iodamoeba williamsi* are commonly seen in human stools, but that trophozoites of the same species are quite rare.

In a recent parasitology manual, by a well-known American author, the contrary statement is made, i.e., that vegetative forms are fairly common in human stools, but that cysts are rare.

This last statement, which I suppose was only a slip of the pen, already corrected in a second printing, called my attention to the need of verifying the correctness of the contrary assumption, which is the one generally held.

As there were in our files the results of a number of fecal examinations, in which we have carefully noted the instances when only cysts were found, only trophozoites, or both, of the different human intestinal protozoa, I thought it might be of some interest to record such results.

The groups providing these data are two. First group (A) comprised 100 purged fecal samples, positives for *I. williamsi*, examined in fresh saline and iodine smears, as well as in hematoxylin-stained slides; a second group (B) comprises 100 normally passed fecal samples,

positive for *I. williamsi,* examined in fresh saline and iodine smears, as well as in iodine smears after zinc sulphate concentration.

The results are as follows:

	(A)	(B)
Number of infections	100	100
Only cysts were found	32	67
Only trophozoites	32	16
Both cysts and trophozoites	36	17

On the basis of the above data, I believe that, in purged fecal samples, without concentration methods, when not only fresh examinations, but also hematoxylin-stained smears, which greatly help in doubtful cases, are made, the chances of finding trophozoites or cysts of this species, are almost equal. When formed stools are examined, without hematoxylin-stained smears, but using the zinc sulphate concentration method, the chance of finding only cysts increases as may be theoretically expected; but even in these conditions, trophozoites alone, or together with cysts, may be found in 33% of the infections, which hardly justifies the statement that they are "rarely" or "very rarely" seen.

I believe Wenyon's statement (Protozoology, 1926, Vol. I, p. 246) that "A remarkable feature of the infections is that often enormous numbers of cysts are passed without there being any indication of the free forms," with the weight given to it not only by the well-known competence of the author, who was one of the first to call attention to the "iodine bodies," but also from its inclusion in one of the best available protozoology text-books, has only been passively followed and rewritten by most authors.

It is my personal opinion that calling the students' attention, as many text-books do, to the fact that trophozoites of *Idodamoeba williamsi* are very rarely seen, results in their frequently overlooking the vegetative forms. The low incidence of this species reported in many surveys, in different localities, may perhaps be explained on such grounds.—ENRIQUE BELTRÁN, *Institute of Public Health and Tropical Diseases (México, D. F.).*

Only two snails were found to harbor *C. nothrica* and both infections apparently were young, since only small numbers of cercariae emerged and most of the sporocysts obtained by cracking the snails were immature.

When not swimming, these cercariae habitually lie on their side or back, with the body strongly flexed, and extend and contract in a peculiar manner that distinguishes them from the other Virgula cercariae studied. Inch-worm movements were never observed in this species.

Description of *Cercaria tranoglandis* sp. nov. (Figs. 12 and 13)

Snail host: Goniobasis livescens (Menke).

Locality: Tippecanoe River, Battle Ground, Indiana.

Specific diagnosis: Body length fully extended 0.173, contracted 0.08; body at medium contraction 0.126 long and 0.076 wide at level of ventral sucker. Tail extended 0.144 long and 0.015 wide at base, contracted 0.036 long and 0.024 wide. Oral sucker 0.038 wide; stylet 0.022 long and 0.005 wide at the base, tip curved ventrally. Virgula organ 0.027 long and 0.027 wide at the anterior end. Ventral sucker in a conspicuous protrusion of the body and 0.016 in diameter. Body and tail finely spinose, spines longer on tip of tail. Papillae with delicate setae scattered over body. Pharynx 0.009 wide, remainder of digestive system not observed. Genital primordium 0.024 wide, C-shaped, slightly posterior to ventral sucker. Three pairs of cephalic glands, the anterior of which possesses very coarse granules. Posterior pair stains darkest with neutral red. Cephalic gland ducts of each side in a single bundle with individual openings at side of stylet tip. Two or more gland-like bodies similar to those of *C. meringura* usually present at pharyngeal level. Excretory bladder broad, U-shaped. Develop in oval-round sporocysts up to 0.2 long in the digestive gland of the snail host.

DISCUSSION

Minute differences between some of the cercariae reported here raises the question as to whether all of them are actually distinct species. The differences observed can be interpreted only as actual specific differences or variations with development in different host species. Life history studies have demonstrated that the cercariae of different species or even genera of adult trematodes may have larvae that are almost indistinguishable. Several examples that could be cited support the view that any constant differences between cercariae, however minute, should be regarded as of at least specific magnitude.

All five species of cercariae described here have the same number of cephalic glands, viz., three pairs. This characteristic immediately distinguishes them from *C. geddesi* Ameel, the only species of the Virgula type described from the U. S. Among the cercariae of that type occurring elsewhere, only the Indian species described by Sewell (1922) and *C. pyramidum* Azim (1936) possess three pairs of cephalic glands. Wesenburg-Lund's (1934) description of *C. nodulosa* von Linstow shows the same number, but with an additional pair of smaller median glands whose ducts do not empty at the stylet tip. Other Virgula cercariae are described as possessing four or more pairs.

The structure of the Virgula organ is essentially the same in all of the five present species, but differs from that described for certain others. Sewell noted a distinction between a bilobed organ with an aperture as in *C. indica* XLII and *C. virgula* Filippi, and a horseshoe-shaped structure that is hollow and with limbs passing backward and dorsally in the manner described for other Virgula cercariae. Since the five present species possess Virgula organs of the bilobed type, this characteristic distinguishes them and *C. indica* XLII from all other Virgula cercariae with three pairs of cephalic glands. From these six species, three, viz., *C. neustica, C. nothrica*

and *C. tranoglandis,* may be separated by the shape of the stylet, the tip of which is curved ventrally. *C. neustica* is peculiar in the small size of its Virgula organ and the medial path of the anterior cephalic gland duct. The chief differences between *C. nothrica* and *C. tranoglandis* are the presence of glandular bodies near the pharynx of *C. tranoglandis* and the more coarsely granular anterior cephalic glands of this species as compared with *C. nothrica.*

In contrast to the species differentiated above, *C. meringura, C. nyxetica* and presumably *C. indica* XLII possess straight stylets, since Sewell (1922) gives no indication to the contrary in his description of the last species. Of these three, *C. meringura* is unique in its large body size, disproportionately long stylet and presence of two pairs of glandular bodies near the pharynx. In *C. nyxetica,* the presence of a rhabdocoel gut, as described for *C. indica* XLII, could not be determined. However, the two are dissimilar in other respects. *C. nyxetica* is characterized by a knobbed stylet, single bundle of cephalic gland ducts on each side, linear cephalic gland arrangement and spinose tail, whereas in *C. indica* XLII, the stylet has only slight shoulders, one pair of cephalic glands and its ducts are mediad, and the tail is aspinose.

In view of the study of Okabe (1937), it seems likely that the Virgula cercariae are larvae of trematodes belonging to the family LECITHODENDRIIDAE. Observations on the metacercarial stages of *C. nyxetica* and ecological relationships of this larva to *Loxogenes bicolor* support this hypothesis.

The family LECITHODENDRIIDAE was created by Odhner (1911) to include two subfamilies, the LECITHODENDRIINAE Looss (1902) and PLEUROGENETINAE Looss (1899). Since that time, there has developed much difference of opinion concerning the intrafamilial relationships of the group, due to lack of agreement concerning taxonomic criteria such as the shape of the bladder, flame cell pattern and position of the vitellaria. A revision of the family was made by Mehra 1935 who reduced the family EUMEGACETIDAE Travassos to the rank of a sub-family and proposed the new sub-families PHANEROPSOLINAE, ANCHITREMINAE, and EXITODENDRIINAE.

Life history studies in other families have cast serious doubt on the validity of many of the adult characters used as a basis for such attempts at classification as those mentioned above. For this reason and because of the lack of information concerning the life histories and embryology of members of the family LECITHODENDRIIDAE, it is believed that there is at present no adequate basis for proposing a natural and hence enduring scheme of classification within the family.

SUMMARY

Five new species of xiphidiocercariae of the Virgula type are described from Indiana, U. S. A. They are *Cercaria meringura* and *C. tranoglandis* from *Goniobasis livescens* (Menke); *C. nothrica* and *C. neustica* from *Pleurocera acuta* Rafinesque; and *C. nyxetica* from *Goniobasis depygis* (Say). A portion of the life history of *C. nyxetica* has been traced and lecithodendriid affinities indicated.

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